Published in final edited form as:

J Phys Chem C Nanomater Interfaces. 2011 September 29; 115(38): 18552–18559. doi:10.1021/jp207047k.

Large Femtosecond Two-Photon Absorption Cross-Sections of Fullerosome Vesicle Nanostructures Derived from Highly Photoresponsive Amphiphilic C₆₀-Light-Harvesting Fluorene Dyad

Min Wang^a, Venkatram Nalla^b, Seaho Jeon^a, Venkatesh Mamidala^b, Wei Ji^{*b}, Loon-Seng Tan^c, Thomas Cooper^c, and Long Y. Chiang^{*a}

^aDepartment of Chemistry, Institute of Nanoscience and Engineering Technology, University of Massachusetts, Lowell, MA 01854, USA

^bDepartment of Physics, National University of Singapore, 2 Science Drive 3, Singapore 117542, Singapore

^cAFRL/RXBN, Air Force Research Laboratory, Wright-Patterson Air Force Base, Dayton, OH 45433, USA

Abstract

We demonstrated ultrafast femtosecond nonlinear optical (NLO) absorption characteristics of bilayered fullerosome vesicle nanostructures derived from molecular self-assembly of amphiphilic oligo(ethylene glycolated) C₆₀-(light-harvesting diphenylaminofluorene antenna). Fullerene conjugates were designed to enhance photoresponse in a femtosecond time scale by applying an isomerizable periconjugation linker between the C₆₀ cage and diphenylaminofluorene antenna subunit in an intramolecular contact distance of only < 3.0 Å. Morphology of C₆₀(>DPAF-EG₁₂C₁)-based fullerosome nanovesicles in H₂O was characterized to consist of a bilayered shell with a sphere diameter of 20–70 nm and a chromophore shell-width of 9.0–10 nm, fitting well with a head-to-head packing configuration of the molecular length. At the estimated effective nanovesicle concentration as low as 5.5×10^{-8} M (molecular molar concentration of 5.0×10^{-4} M) in H₂O, two-photon absorption (2PA) phenomena were found to be the dominating photophysical events showing a large molar concentration-insensitive 2PA cross-section value equivalent to 8500 GM in a form of nanovesicles, on average. The observed NLO characteristics led to a sharp trend of efficient light-transmittance intensity reduction at the input laser intensity above 100 GW/cm².

Keywords

C₆₀ monoadduct; fullerosome; nonlinear optical absorption; nanovesicle; light-transmittance reduction; 2PA cross-section

*To whom correspondence should be addressed. (W.J.) phyjiwei@nus.edu.sg; (L.Y.C.) Long_Chiang@uml.edu.

Supporting Information Available: Synthetic procedure of the compound C₆₀(>DPAF-C₂M) **1** and ¹H NMR spectra of **1**, C₆₀(>DPAF-OH) **2**, and C₆₀(>DPAF-EG₁₂C₁) **4**. This information is available free of charge via the internet at <http://pubs.acs.org>.

Introduction

Nonlinear photonic behavior and the corresponding photophysical properties of complex fullerene derivatives and other organic chromophores¹ under intense light irradiation involve both simultaneous multiphoton absorption (MPA) and excited state-based reverse saturable absorption (RSA). The phenomena of RSA are related to the well-recognized fact that excited states of C₆₀ are more polarizable with larger absorption cross-sections than those of the ground states. Nearly co-existence of fullereryl singlet and triplet excited states fits well with the required facile generation of populated excited states for obtaining strong nonlinear optical (NLO) responses² and the enhanced excited state absorption.^{3,4} These RSA properties have been applied as the fundamental principle in the development of effective materials for protection from laser pulses in high-intensity in the past decade.⁵⁻¹⁷ Nonlinear absorption behavior of C₆₀ and its derivatives appeared to be dominated by the excited-state RSA in visible range and the two-photon absorption (2PA) process in the NIRIR region.^{18,19} Particular interests in the use of NLO effects in materials application include the development of efficient light intensity attenuators for preventing damage against high-intensity irradiation and two-photon absorption based photodynamic therapy (2γ-PDT).

Nonlinear MPA efficiency of fullerene-chromophore derivatives was observed to be structure-dependent and concentration-dependent leading to the variation of 2PA cross-section (σ_2) values and nonlinear laser intensity transmittance capability.²⁰ In high concentration ($>5.0 \times 10^{-3}$ – 10^{-2} M) cases, solid aggregation of C₆₀-derived chromophores may restrict both MPA and RSA photoprocesses due to improper access of internally packed molecules in the particle to the activation light source. Below this concentration, a certain degree of irregular molecular coalescence into nanoparticles is also feasible. As the size of coalescence increases, light absorption by the fullerene molecules located at the surface area limits penetration of the light deep into the particle. Aggregation tendency of fullerene derivatives is obvious due to their strong ability to form a tight cage packing via π -electron-induced hydrophobic–hydrophobic interactions. This is consistent with our observation²⁰ of increasingly high 2PA cross-section values at low concentrations below 10^{-4} M showing reduced aggregation sizes in TEM micrographs. However, below this concentration range, it is too dilute to achieve sufficient transmittance attenuation efficiency. Accordingly, we proposed a resolution by using well-organized self-assembly of fullerene derivatives into bilayered nanovesicles, each in a diameter of 20–50 nanometers, for enhancing NLO properties at a relatively low molar concentration range of 10^{-4} M. The design should increase the local light absorption on a condensed molecular nanostructure with the possibility of each C₆₀-chromophore molecule having appropriate exposure to the incident light source. In principle, a controllable bilayer nanovesicle thickness of 5.0–10 nm should be thin enough to allow full light penetration through the membrane. This will lead to NLO responses and characters, including 2PA cross-sections and light-transmittance reduction efficiency, resembling those of well-solubilized fullerene derivatives in solution.

To prove the concept, we designed and synthesized amphiphilic C₆₀-light-harvesting fluorene antenna conjugates as C₆₀(>DPAF-EG_x) derivatives with a proportional weight-ratio balance between the hydrophobic and hydrophilic moieties for the facile vesicle formation in aqueous solution. In this report, we described the modified synthesis,²¹ characterization, and concentration-dependent NLO responses of C₆₀(>DPAF-EG₁₂C₁)-derived nanovesicles in femtosecond region. The observed NLO behavior was correlated to the ability of these vesicles in reducing the intensity of transmitted light.

Experimental Section

Synthesis of C₆₀-methanocarbonyl-9,9-diethanol-2-diphenylaminofluorene 2, C₆₀(>DPAF-OH)

In a round-bottom flask was added C₆₀(>DPAF-C_{2M})²¹ (**1** of Scheme 1, 0.26 g, 0.22 mmol), trimethyl iododisilane (0.2 g, 1.0 mmol), pyridine (0.1 mL), and dried chloroform (50 mL) followed by reflux under an inert atmosphere (N₂) for a period of 72 h. After the disappearance of the starting material was confirmed by the spot check on analytical thin-layer chromatographic (TLC) plate, the reaction mixture was cooled to room temperature and added methanol (5.0 mL). It was stirred for an additional 1.0 h. The organic layer of the reaction mixture was washed sequentially with saturated sodium bicarbonate (25 mL) and water (2 × 25 mL), followed by drying the solution over magnesium sulfate and subsequently removing the solvent *in vacuo*. The resulting crude brown solid was purified by column chromatography using silica gel as the stationary phase and toluene–ethylacetate (3:2) as the eluent to afford C₆₀-methanocarbonyl-9,9-diethanol-2-diphenylaminofluorene **2**, C₆₀(>DPAF-OH) in ~60% yield (0.15 g.). Spectroscopic data of the compound **2**: FT-IR (KBr) $\nu_{\max}$ 3441 (s), 2955 (w), 2922 (m), 2852 (m), 1665 (s), 1647 (s), 1590 (s), 1489 (s), 1466 (s), 1420 (m), 1274 (m), 1121 (w), 1030 (w), 814 (w), 752 (s), 696 (s), 573 (w), and 526 (s) cm⁻¹; UV–vis (CHCl₃, cutoff at 245 nm, 1.0 × 10⁻⁵ M) $\lambda_{\max}$ 256, 322, 409, and 692 nm; ¹H NMR (500 MHz, CDCl₃, ppm) δ 8.53 (d, 1H), 8.44 (s, 1H), 7.86 (d, 1H), 7.68 (d, 1H), 7.33–7.30 (m, 2H), 7.21–7.09 (m, 10H), 5.70 (s, 1H), 3.16 (m, 4H), and 2.46–2.28 (m, 4H). Hydroxyl group peak appeared as a singlet at δ 1.62 and confirmed by D₂O exchange.

Synthesis of C₆₀-methanocarbonyl-9,9-dipolyethyleneglycol-2-diphenylaminofluorene 4, C₆₀(>DPAF-EG₁₂C₁)

Polyethylene glycol 600 diacid (0.46 g, 0.08 mmol) was taken in a 100 mL round-bottom flask and stirred under reduced pressure to remove residual water for a period of 5.0 h in an oil bath maintained at 80 °C. The flask was then cooled to room temperature and added DCC (0.16 g, 0.08 mmol), DMAP (0.09 g, 0.08 mmol), C₆₀(>DPAF-OH) (**2**, 0.15 g, 0.013 mmol), and anhydrous 1,2-dichloroethane (50 mL). The solution was stirred in an oil bath maintained at 65 °C for 2.0 h. After the spot check of the reaction mixture on an analytical TLC plate showing the complete disappearance of the starting material, it was added methanol (1.0 mL) followed by stirring for an additional 2.0 h. At the end of the reaction, solids in the solution were filtered off and the filtrate dried on rotavapor. The resulting crude product was dissolved in THF–H₂O (1:1, 10 mL) and dialyzed against distilled water using a dialysis membrane of MWCO 1000 to ensure complete removal of unreacted polyethylene glycol. The product **4**, C₆₀(>DPAF-EG₁₂C₁), was obtained as semisolids in 40% yield after the removal of water using the freeze-dry evaporation method. Spectroscopic data of the compound **4**: FT-IR (KBr) $\nu_{\max}$ 3435 (s), 3329 (s), 2926 (s), 2970 (s), 1751 (s), 1670 (m), 1644 (s), 1592 (s), 1452 (s), 1384 (s), 1279 (w), 1244 (w), 1200 (w), 1117 (s), 947 (m), 882 (m), 846 (m), 761 (m), 701 (s), and 526 (s) cm⁻¹; UV–vis (CHCl₃, cut-off at 245 nm, 1.0 × 10⁻⁵ M) $\lambda_{\max}$ 256, 322, and 409 nm; ¹H NMR (500 MHz, CDCl₃, ppm) δ 8.57 (d, 1H), 8.39 (s, 1H), 7.84 (d, 1H), 7.63 (d, 1H), 7.36–7.10 (m, 12H), 5.73 (s, 1H), 4.30 (t, 4H), 4.20 (s, 4H), 3.70 (s, 4 H), 3.62 (broad, 80 H), 3.38 (s, 3H), 3.26 (s, 3H), and 2.60–2.37 (m, 4 H).

Preparation of C₆₀(>DPAF-EG₁₂C₁)-derived nanovesicles

The compound C₆₀(>DPAF-EG₁₂C₁) **4** (1.4 mg) was dissolved in THF–DMSO (1:1, 0.1 mL) under ultrasonication for *ca.* 5.0 min to enhance dissolution. It was added H₂O (1.9 mL) under vigorous stirring to give a solution of 3.0 × 10⁻⁴ M in concentration. A portion of this solution was diluted by H₂O to a defined concentration for subsequent studies.

Results and Discussion

We demonstrated recently the feasibility of achieving ultrafast linear and nonlinear photoresponses in inducing intramolecular energy- and/or electron-transfer from the diphenylaminofluorene donor antenna moiety to the C_{60} acceptor moiety by attaching one or multiple hindered light-harvesting 9,9-dialkyl-2-diphenylaminofluorenyl (DPAF- C_n) fluorescent chromophore subunit to a methanofullerene ($C_{60}>$) cage in a periconjugation manner leading to C_{60} -(antenna) $_x$ analogues nanostructures, such as $C_{60}>$ (DPAF- C_n) $_x$ ($x = 1, 2, \text{ or } 4$).^{17,20} Thus, it is of our interest in incorporating a similar acceptor-donor antenna component of C_{60} -DPAF for this study with the replacement of hydrophobic alkyl side-chains by highly hydrophilic oligo(ethylene glycol) (EG_n) giving $C_{60}>$ (DPAF- EG_n D) **3** and $C_{60}>$ (DPAF- EG_nC_1) **4**, as shown in Scheme 1, to increase the amphiphilicity of the molecule. The chain length of EG_n arms was selected to match with the overall molecular radius and to achieve a balance between the hydrophobicity and hydrophilicity of resulting molecules. This facilitates their high efficiency in the organization of molecular self-assembly in aqueous solution.

Experimentally, synthesis of amphiphilic 9,9-di[methoxycarboxymethyl oligo(ethylene glycol)-methylcarboxyethyl]-2-diphenylaminofluorenyl-methano- [60]fullerene $C_{60}>$ (DPAF- $EG_{12}C_1$) **4** was carried out from an intermediate compound 9,9-di(methoxyethyl)-2-diphenylaminofluorenyl-methano[60]fullerene $C_{60}>$ (DPAF- C_{2M}) **1** by a modified literature procedure.²¹ Several reagents and conditions were evaluated for demethylation reaction of the complex molecule **1** that allowed us to conclude the best method, giving a yield of >90%, using trimethylsilyl iodide as the ether silylation reagent followed by hydrolysis of the resulting silyloxy moiety, as shown in Scheme 1. The corresponding di(hydroxyethyl) fullerenyl intermediate $C_{60}>$ (DPAF-OH) **2** was then subjected to the reaction with oligo(ethylene glycol) bis(carboxymethyl) ether (molecular weight 600, EG_{12}) in the presence of 1,3-dicyclohexylcarbodiimide (DCC, 2.2 equiv. per EG_{12}) and 4-dimethylaminopyridine (DMAP) at room temperature. Completion of the reaction was monitored by the disappearance of **2** on a thin-layer chromatographic plate (TLC) to afford the tetraester intermediate product $C_{60}>$ (DPAF- $EG_{12}D$) **3**. Subsequent methylation of **3** was performed by the addition of methanol to give $C_{60}>$ (DPAF- $EG_{12}C_1$) **4**. Removal of an excess of EG_{12} from the product was made by dialysis of **4** against distilled water using a dialysis membrane (molecular weight cut-off at 1,000). It was followed by the water removal using the freeze-dry evaporation technique.

Structural characterization of **4** was made by various spectroscopic techniques. It was made firmly based on the X-ray single crystal structural analysis of $C_{60}>$ (DPAF- C_{2M}) **1** giving unambiguous verification of the main dimethoxyethylated chromophore moiety.²¹ Conversion of **1** to the corresponding dihydroxyethylated derivative **2** was confirmed by the disappearance of singlet methoxy proton peak at the chemical shift δ 3.05 with slight upfield-shift of ethyl proton peaks to δ 3.14 from δ 2.85 and a new hydroxyl proton peak appearing as a singlet at δ 1.72, as substantiated by D_2O exchange. Attachment of two EG_{12} groups on **2** followed by the methylation reaction leading to the formation of **3** and **4**, respectively, in sequence can be easily substantiated by the detection of ester carbonyl functional moieties via an absorption band appearing at 1750.7 cm^{-1} in the Infrared spectrum of **4** and two peaks at δ 172.2 and 170.4 corresponding to the chemical shift of two types of ester carbons in ^{13}C NMR spectrum.

Strong hydrophobic interactions and association among fullerene cages serve as the major attractive driving force for amphiphilic fullerene derivatives, such as **4**, to organize into an ordered shape and form in aqueous solution that resulted in efficient molecular assembly. In close resemblance to small amphiphilic surfactant molecules in forming micelle structures in

water, [60]fullerenyl amphiphilics exhibit high tendency in forming a hydrophobic bilayer shell that consists of an array of C_{60} cages in a submicrospheric membrane, below the critical aggregation concentration, known as buckysome or fullerosome vesicles.²²⁻²⁶ Above the critical aggregation concentration, formation of multi-bilayered vesicles is also possible. In the molecular structure of $C_{60}(>>DPAF-EG_{12}C_1)$, the moiety of $C_{60}>$ and DPAF can be regarded as water-insoluble chromophore units that give irregular packing in the solid particle due to mismatched moiety shapes. Competitive intermolecular interaction force among like-moieties $C_{60}-C_{60}$ and DPAF-DPAF will govern the molecular self-assembly character of **4**. Therefore, to maximize the packing order among $C_{60}>$ moieties, we dissolved $C_{60}(>>DPAF-EG_{12}C_1)$ in a minimum amount of water-miscible organic solvent, such as THF-DMSO (1:1, v/v), to dissociate each C_{60} -DPAF chromophore unit from each other under the ultrasonication condition prior to its addition into water. Initiation of molecular self-assembly was assisted by continuing ultrasonication for few minutes in aqueous solution. The form and size of self-organization may vary upon modification of the preparation procedure and the subsequent treatment. Therefore, to make it consistent for all samples, a master solution was prepared at 5.0×10^{-4} M in concentration that was then diluted afterward to 1.0×10^{-4} , 5.0×10^{-5} , or 10^{-6} M for the transmission electron microscopy (TEM) and femtosecond Z-scan measurements.

Carbon-copper film grids in a 200-mesh size were used for the topography investigation of molecularly assembled structures, derived from $C_{60}(>>DPAF-EG_{12}C_1)$, by TEM images. Samples were prepared by coating the grid with a solution of **4** at 1.0×10^{-6} M diluted from the master nanovesicle solution (5.0×10^{-4} M in H_2O), followed by the freeze-dry technique under vacuum to retain to the vesicle shape on the grid. Consistent TEM microimages of nanovesicles were obtained and revealed by many regular bilayer spheres in a size ranging from 20 to 70 nm in diameter, as shown in Figure 1a. Majority of the spheres were larger than the typical micelle size of 20–30 nm in diameter derived from lipid molecules. Interestingly, no obvious large solid particles were detected even though this sample solution was not filtered through a filter membrane of 0.45 μ m in pore size that we normally followed as a procedure. Morphology of the vesicle shell membrane was revealed by a dark ring area with a roughly even wall-width and a smooth shape around the ring image. Measurement of the vesicle wall thickness, as shown in Figure 1b marked by the cross-ended lines, gave a wall-width of roughly 9.0–10 nm. This length fits approximately with the linear bilayer molecular dimension of $C_{60}(>>DPAF-EG_{12}C_1)$ in 16–18 and 7.0–8.0 nm, estimated by the 3D molecular structural modeling of **4** with a fully stretched oligo(ethylene glycol) chain and chromophore core width, respectively, as shown in Figure 2. This implied a shell-like bilayer consisting of a head-to-head packing of $C_{60}(>>DPAF)$ chromophore moieties in the nanovesicle membrane region. It is also consistent with the X-ray single crystal structure of the related analogous $C_{60}(>>CPAF-C_2)$ showing a highly ordered array of $C_{60}>$ cages in the unit cell packing in the presence of similar planar diphenylaminofluorene chromophores.²⁷

Nonlinear optical properties of the nanovesicle solution were measured by using the femtosecond Z-scan technique. The femtosecond laser pulses were generated by a mode-locked Ti:Sapphire laser (Quantronix, IMRA), which seeded a Ti:sapphire regenerative amplifier (Quantronix, Titan) with the wavelength of 780 nm, the pulse width of 500 fs, and the repetition rate of 1.0 kHz for the Z-scan experiment. During the data collection, laser pulses with a beam waist of ~ 30 μ m were focused onto the vesicle solution in a quartz cuvette having 1.0-mm-path length. The incident and transmitted laser powers were monitored as the cuvette moved along the Z-direction toward and away from the focus position. The same setup was applied for femtosecond irradiance-dependent transmission experiments.

The measured 2PA efficiency of **4** can be correlated to the energy diagram proposed in Figure 3. Both highly fluorescent DPAF donor and C₆₀> acceptor segments are two-photon absorptive at the irradiation wavelength of 780 nm with the former component exhibiting stronger optical absorption coefficient at this particular wavelength, making it a main light-harvesting antenna unit in **4** for enhancing nonlinear photoactivity. Periconjugation linkage between these two moieties incurs ultrafast intramolecular energy or electron-transfer processes going from photoexcited ¹(DPAF)*-EG₁₂C₁ to C₆₀>, resembling that of the recent report using C₆₀(>DPAF-C_n) as the model of demonstration.¹⁷ The photoresponsive time scale of <130 fs fits well in the current femtosecond studies in coupling with the rich excited-state absorption (ESA) of fullerene cage. This intramolecular transfer mechanism is plausible due to, for example, the lowest excited singlet energy of the similar chromophore unit in C₆₀(>¹DPAF*-C_n) and ¹C₆₀*(>DPAF-C_n) being estimated to be 2.74 (452 nm) and 1.74 (714 nm) eV, respectively, based on steady-state fluorescence measurements.²⁸ As the 2PA and ESA of both DPAF-EG₁₂C₁ and C₆₀> moieties occur in a nearly concurrent event, large enhancement of the overall NLO response capability of C₆₀(>DPAF-EG₁₂C₁) nanomaterials should be achievable in the ultrashort time scale of femtoseconds.

Intensity-dependent open-aperture Z-scans were carried out under the irradiance intensity of either 45, 155, or 300 GW/cm² on the samples of C₆₀(>DPAF-EG₁₂C₁) at three molecular molar concentrations of 5.0 × 10⁻⁵, 1.0 × 10⁻⁴, and 5.0 × 10⁻⁴ M. The sample solution was prepared by dissolving **4** in a minimum amount of THF–DMSO (1:1, v/v) prior to the addition of deionized water into a master solution in a similar procedure as that applied for the nanovesicle formation in TEM measurements for the direct data correlation. It was then diluted with H₂O into an appropriate concentration for the Z-scan experiments. Steady-state visible absorption spectra of these vesicle solutions were shown in Figure 4. A weak broad characteristic absorption band of the fullerenyl π-electron system of the C₆₀ cage moiety centered at 700–720 nm was not visible, perhaps, due to broadening as a result of its packing in a membrane structure. The absorption band in the region of 400–450 nm was attributed to the DPAF-EG₁₂C₁ moiety. Intensity increase of this band corresponds roughly in proportion to the increase of concentration from 5.0 × 10⁻⁵ to 5.0 × 10⁻⁴ M. A Negligible shift between these three absorption peak maxima of Figures 4a–4c was indicative of no clear packing orientation change of DPAF chromophore moiety during the solution dilution. At the higher concentration of 5.0 × 10⁻⁴ M, the profile showed a low linear optical absorbance at 780 nm. Molecular packing in a nanovesicle form is expected to result in large broadening of the absorption edge at long wavelengths

Experimentally, the collected open-aperture Z-scan data sets were normalized to the linear transmittance and sample inhomogeneities for all scans by the correction of the background transmittance, $T(|Z| \gg Z_0)$. The normalized transmittance $\Delta T(Z)$ was expressed as $T(Z)/T(|Z| \gg Z_0)$. Accordingly, the change in the normalized transmittance is indicative of the nonlinear (or light-fluence-dependent) part in the sample's absorption. To quantify and differentiate both phenomena of the saturation absorption (SA) and the 2PA, we employed the following general expression for the entire absorption coefficient of the vesicle solution:

$$\begin{aligned} \frac{dI}{dz} &= \frac{\sigma_0 N_0 I}{\sqrt{1+I/I_s}} \sigma_1 N_1 I \beta I^2 \\ \text{with } I &= I_{00} \left[\frac{\omega_0^2}{\omega^2(z)} \right] \exp\left(-\frac{r^2}{r_p^2}\right) \exp\left[-\frac{2r^2}{\omega^2(z)}\right] \\ \text{and } \omega(z) &= \omega_0 \left\{ 1 + \left(\frac{z}{z_0} \right)^2 \right\}^{\frac{1}{2}}; z_0 = \frac{\pi \omega_0^2}{\lambda} \end{aligned} \quad (1)$$

where σ_0 is the effective ground-state absorption cross-section, σ_1 is the excited-state absorption cross-section from S₁ state, β is the 2PA coefficient, N_0 and N_1 are the populations in S₀ and S₁ states, respectively, as defined in Figure 3. z_0 is the Rayleigh range,

ω_0 is the beam waist at the focus point, and τ_p is the input pulse width used. I_s is the saturation intensity, I is the intensity as a function of r , t , and z , and I_{00} is the peak intensity at the focus point of the Gaussian beam. In the denominator of the first term on the left side of Eqn. (1), the square root is used to reflect the inhomogeneous nature of our system. The above differential equation was first solved together with rate equations for excited-state populations. It was then integrated over the time and length along the radial direction, and solved numerically using the Runge-Kutta fourth order method. Assuming the input light beam to be a Gaussian, the limits of integration for r , t , and z were varied from 0 to ∞ , $-\infty$ to ∞ , and 0 to L (length of the sample), respectively.

It is important to notice that the term of $\sigma_0 N_0$ is an effective linear absorption coefficient of the sample. It should be the ground-state absorption of the solvent when the sample contains zero or extremely low concentration of $C_{60}(>>DPAF-EG_{12}C_1)$. On the other hand, the term is dominated the ground-state absorption of $C_{60}(>>DPAF-EG_{12}C_1)$ when its concentration is so high that its ground-state absorption is much greater than that of the solvent.

By applying Eqn. (1) to fit the Z-scans, we first found that the ESA term, $\sigma_1 N_1$, be insignificant. As such, Eqn. (1) is reduced to

$$\frac{dI}{dz} = \frac{\sigma_0 N_0 I}{\sqrt{1 + I/I_s}} \beta I^2 \quad (2)$$

Based on the best fittings, we unambiguously extracted the values of two parameters: I_s and β . From the latter, the 2PA cross-section values (σ_2) can be calculated by the formula $\sigma_2 = \beta \hbar \omega / N$, where $\hbar \omega$ is the photon energy and N is the number of molecules.

Combinative effects induced by both SA and 2PA at femtosecond excitations were displayed in Figure 5. Reference solvent mixture [THF–DMSO–H₂O (1:1:38, v/v)] scans (Figure 5a) were taken for the purpose of comparison and calibration. The trace profile showed a slightly increased transmittance at $Z = 0$ above the normalized baseline indicating the existence of absorption saturation of this solvent mixture at the light intensity of 155 and 300 GW/cm². The quantity of transmittance increase remained in a similar range with the solution containing $C_{60}(>>DPAF-EG_{12}C_1)$ at the molecular molar concentration of 5.0×10^{-5} M (Figure 5b). However, it was accompanied with a systematic raise in response to the increase of applied light intensity from 45 to 300 GW/cm², giving clear SA phenomena in correlation to that of the solvent mixture in Figure 5a. The rationale was given by the fact that the total number of molecule **4** at this concentration estimated for covering the surface area of nanovesicles, having an average diameter of ~45 nm (Figure 1), should lead to an effective vesicle concentration of 5.5×10^{-9} MV by the approximation. It was calculated by packing C_{60} cages, each with 1.0 nm in diameter, to cover the entire surface area of vesicle sphere that resulted in a total of ~9000 $C_{60}(>>DPAF-EG_{12}C_1)$ molecules per bilayered thickness. It is rather small for the NLO signal detection under the current experimental conditions that allowed the domination of saturation absorption arising from the solvent mixture.

As the effective nanovesicle concentration increased to 1.1×10^{-8} MV (molecular molar concentration of 1.0×10^{-4} M, Figure 5c), positive signs for absorptive nonlinearities in Z-scan trace profiles began to appear at the applied light intensity of 155 and 300 GW/cm². It showed systematic decrease of the light-transmittance in response to the increase of irradiance intensity. These multiphoton absorption phenomena became significant with a large decrease in transmittance when the effective nanovesicle concentration being increased to 5.5×10^{-8} MV (molecular molar concentration of 5.0×10^{-4} M, Figure 5d), indicating clearly the dominance of 2PA at this vesicle concentration.

From curve fittings with Eqn. (1), the value of two parameters, saturation intensity (I_s) and 2PA coefficient (β), was obtained and plotted in Figure 6, showing an approximately linear relationship of the increasing β value to the increasing sample concentration with the monotonically declining trend of the I_s value. The decline in the I_s value of the sample is anticipated because it characterizes the saturation intensity of the solvent when the concentration of $C_{60}>DPAF-EG_{12}C_1$ is extremely low. As the $C_{60}>DPAF-EG_{12}C_1$ concentration increases to 5.0×10^{-4} M, however, the measured I_s value is dominated by the saturation characteristics of $C_{60}>DPAF-EG_{12}C_1$ molecules.

As shown in Figure 6, the β values were given as 0.001, 0.0025, and 0.011 cm/GW for the molar sample concentration of 5.0×10^{-5} , 1.0×10^{-4} , and 5.0×10^{-4} M, respectively. The corresponding 2PA cross-section values (σ_2) calculated for **4**-derived nanovesicle solutions were found to be insensitive to the molar concentration with the average value of 85×10^{-48} cm⁴ sec photon⁻¹ molecule⁻¹ or 8500 GM, as shown in Figure 7. By using the previously measured σ_2 value of hydrophobic $C_{60}>DPAF-C_9$ as 21.9×10^{-48} cm⁴ sec photon⁻¹ molecule⁻¹ (or 2190 GM, 1.0×10^{-4} M in CS₂)²⁰ in 160-fs region as the molecular reference for comparison in this study, we proposed that the observed NLO properties arise from the two-photon absorption of the DPAF moiety as the main photo-event, forming the corresponding transient $C_{60}>^1DPAF^*-C_9$ state followed by the intramolecular energy-transfer process to $^1C_{60}^*>DPAF-C_9$ state. The chromophore component of $C_{60}>DPAF-C_9$ is identical to that of **4**. Accordingly, we expect the similar photophysical transient mechanism occurring in the nanovesicle structure of $C_{60}>DPAF-EG_{12}C_1$. The argument is valid due to the fact of much lower nonlinear $C_{60}>$ cage absorption at 780 nm as compared with that of the DPAF moiety.

Nonlinear optical transmittance attenuation properties of **4**-derived nanovesicle solutions in 1.0-mm thickness were investigated by irradiance-dependent transmission measurements at the wavelength of 780 nm. The same setup as that used in the Z-scan studies conducted by 500-fs laser pulses was applied, except the sample being fixed at the focus area when the incident pulse intensity was varied. The results collected at three concentrations were illustrated in Figure 8, where Eqn. (2) was used to fit the measured intensity-dependent transmittances. The obtained values for two parameters, I_s and β , were in good agreement with those derived from the fitting of Z-scans.

No nonlinear light-transmittance intensity reduction was detected on the solvent mixture (Figure 8, square) that provided the baseline for comparison with all nanovesicle sample solutions. In the case of the vesicle solution in a molar concentration of 5.0×10^{-5} M, light transmittance efficiency increases slightly at all irradiance intensities (Figure 8, ring). This is consistent with the observed dominance of absorption saturation stated above at this concentration. When the molecular molar concentration of the solution being increased to 1.0×10^{-4} M, a slight improvement of light-transmittance reduction was detected with the irradiance intensity going above 200 GW/cm². Most interestingly, a rapid downward decreasing trend of the normalized transmittance was observed for the nanovesicle solution in a molecular molar concentration of 5.0×10^{-4} M with the input light intensity above 100 GW/cm². This deviation from the linear transmission line with a sharp decline indicated the initiation of 2PA event and efficient limiting effect. Significant enhancement in lowering the transmittance can be correlated to the high 2PA cross-sections of $C_{60}>DPAF-EG_{12}C_1$ -derived nanovesicle at this concentration

Conclusion

We demonstrated ultrafast femtosecond nonlinear optical (NLO) absorption characteristics of bilayered fullerosome nanovesicles derived from molecular self-assembly of amphiphilic

oligo(ethylene glycolated) C₆₀-(light-harvesting fluorene antenna) nanostructures. Effective NLO behavior of these nanomaterials arises from the facile ultrafast intramolecular electron-/or energy-transfer from the photoexcited diphenylaminofluorene antenna donor to the C₆₀ acceptor cage in femtoseconds (~130 fs).¹⁷ Molecular self-assembly of one such example as C₆₀(>DPAF-EG₁₂C₁) in H₂O gave the formation of bilayered fullerosome nanovesicles. As a result, two-photon absorption phenomena were found to be the dominating photophysical events showing a large molar concentration-insensitive 2PA cross-section value equivalent to 8500 GM in a form of vesicles, on average, at the estimated effective nanovesicle concentration as low as 5.5×10^{-8} MV (molecular molar concentration of 5.0×10^{-4} M) in H₂O. The observed NLO characteristics led to a sharp trend of efficient light-transmittance intensity reduction at the input laser intensity above 100 GW/cm².

These results led to our conclusion that, in a form of bilayered fullerosome, facile light exposure of each C₆₀-diphenylaminofluorene antenna conjugate molecule in the membrane should be plausible to *concurrent* photoexcitation. As it partially bears resemblance to similar phenomena in dilute solution, the event may significantly reduce the number of ground state C₆₀ cage moiety and minimize the potential self-quenching effect. Furthermore, the other possible factor leading to the NLO efficiency loss via bimolecular triplet-triplet annihilation processes of ³(C₆₀>)* in the aggregated form of fullerene monoadducts^{29,30} will not be present in the current femtosecond study. It is reasoned by our previous observation of the effective ³(C₆₀>)* formation in similar C₆₀(>DPAF-C_n) analogous molecules upon photoexcitation at a longer time scale of nanoseconds (~1.4 ns).¹⁷ Accordingly, the use of fullerosomes may represent a suitable approach toward potential solid film coating applications of nanomaterials for ultrafast NLO photoresponses and mass drug-delivery of two-photon-based photosensitizers for photodynamic therapeutic (2γ-PDT) treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors at UML thank the financial support of Air Force Office of Scientific Research (AFOSR) under the grant number FA9550-09-1-0380 and FA9550-09-1-0183 and National Institute of Health (NIH) under the grant number 1R01CA137108. The authors are also grateful to the financial support from the National University of Singapore under the research Grant number R-144-000-213-112.

References

1. For a recent review. He GS, Tan L-S, Zheng Q, Prasad PN. Chem Rev. 2008; 108:1245–1330. [PubMed: 18361528]
2. Zhou QL, Heflin JR, Zamani-Khamiri KY, Garito A. Phys Rev A. 1991; 43:1673–1676. [PubMed: 9905207]
3. Tutt LW, Kost A. Nature. 1992; 356:225–226.
4. Kost A, Tutt LW, Klein MB, Dougherty TK, Elias WE. Opt Lett. 1993; 18:334–336. [PubMed: 19802127]
5. Henari FZ, Cazzini KH, Weldon DN, Blau WJ. Appl Phys Lett. 1996; 68:619–621.
6. Sun Y-P, Riggs JE. Int Rev Phys Chem. 1999; 18:43–90.
7. Riggs JE, Sun Y-P. J Phys Chem A. 1999; 103:485–495.
8. Song YL, Fang GY, Wang YX, Liu ST, Li CF, Song LC, Zhu YH, Hu QM. Appl Phys Lett. 1999; 74:332–334.
9. Maggini M, Faveri CD, Scorrano G, Prato M, Brusatin G, Guglielmi M, Meneghetti M, Signorini R, Bozio R. Chem Eur J. 1999; 5:2501–2510.

10. Dou K, Knobbe ET. *J Nonlin Opt Phys Mater*. 2000; 9:269–287.
11. Chiang LY, Padmawar PA, Canteewala T, Tan L-S, He CS, Kanna R, Vaia R, Lin T-C, Zheng Q, Prasad PN. *Chem Commun*. 2002:1854–1855.
12. Koudoumas E, Konstantaki M, Mavromanolakis A, Couris S, Fanti M, Zerbetto F, Kordatos K, Prato M. *Chem Eur J*. 2003; 9:1529–1534.
13. Padmawar PA, Canteenwala T, Verma S, Tan L-S, Chiang LY. *J Macromol Sci A, Pure Appl Chem*. 2004; 41:1387–1400.
14. Kopitkovas G, Chugreev A, Nierengarten JF, Rio Y, Rehspringer JL, Honerlage B. *Opt Mater*. 2004; 27:285–291.
15. Elim HI, Quyang J, Goh SH, Ji W. *Thin Solid Films*. 2005; 477:63–72.
16. Padmawar PA, Canteenwala T, Tan L-S, Chiang LY. *J Mater Chem*. 2006; 16:1366–1378.
17. Padmawar PA, Rogers JO, He GS, Chiang LY, Canteenwala T, Tan L-S, Zheng Q, Lu C, Slagle JE, Danilov E, McLean DG, Fleitz PA, Prasad PN. *Chem Mater*. 2006; 18:4065–4074.
18. Rao SV, Rao DN, Akkara JA, DeCristofano BS, Rao DV. *Chem Phys Lett*. 1998; 297:491–498.
19. Banfi G, Fortusini D, Bellini M, Milani P. *Phys Rev B: Condensed Matter*. 1997; 56:R10075–R10078.
20. Elim HI, Anandakathir R, Jakubiak R, Chiang LY, Ji W, Tan LS. *J Mater Chem*. 2007; 17:1826–1838.
21. Verma S, Hauck T, El-Khouly ME, Padmawar PA, Canteenwala T, Pritzker K, Ito O, Chiang LY. *Langmuir*. 2005; 21:3267–3272. [PubMed: 15807563]
22. Sawamura M, Nagahama N, Toganoh M, Hackler UE, Isobe H, Nakamura E, Zhou S, Chu B. *Chem Lett*. 2000:1098–1099.
23. Brettreich M, Burghardi S, Bottcher C, Bayerl T, Bayerl S, Hirsch A. *Angew Chem Int Ed*. 2000; 39:1845–1848.
24. Zhou S, Burger C, Chu B, Sawamura M, Nagahama N, Toganoh M, Hackler UE, Isobe H, Nakamura E. *Science*. 2001; 291:1944–1947. [PubMed: 11239150]
25. Cassell AM, Asplund CL, Tour JM. *Angew Chem Int Ed*. 1999; 38:2403–2405.
26. Sano M, Oishi K, Ishi-I T, Shinkai S. *Langmuir*. 2000; 16:3773–3776.
27. Chiang LY, Padmawar PA, Rogers-Haley JE, So G, Canteenwala T, Thota S, Tan L-S, Pritzker K, Huang Y-Y, Sharma SK, Kurup DB, Hamblin MR, Wilson B, Urbas A. *J Mater Chem*. 2010; 20:5280–5293. [PubMed: 20890406]
28. Luo H, Fujitsuka M, Ito O, Padmawar P, Chiang LY. *J Phys Chem B*. 2003; 107:9312–9318.
29. Riggs JE, Sun YP. *J Phys Chem A*. 1999; 103:485–495.
30. Sudeep PK, James PV, Thomas KG, Kamat PV. *J Phys Chem A*. 2006; 110:5642–5649. [PubMed: 16640358]

Figure 1.

TEM micrographs of $C_{60}(>DPAF-EG_{12}C_1)$ **4**-derived bilayer vesicles prepared at a concentration of 5.0×10^{-4} M in H_2O , followed by dilution to 10^{-6} M for the carbon grid coating, showing (a) the edge of the bilayer shell and (b) the enlarged vesicle shell dimension in the inset.

Figure 2. Schematic presentation of the segment membrane structure of a bilayered nanovesicle derived from C₆₀(>DPAF-EG₁₂C₁).

Figure 3. Energy diagram of the donor antenna and acceptor moieties of $\text{C}_{60}>\text{DPAF-EG}_{12}\text{C}_1$ showing ultrafast intramolecular energy-transfer¹⁷ in femtosecond experiments.

Figure 4. Steady-state visible absorbance spectra of C₆₀(>DPAF-EG₁₂C₁)-derived nanovesicle solution in deionized H₂O at concentrations of (a) 5.0 × 10⁻⁵, (b) 1.0 × 10⁻⁴, and (c) 5.0 × 10⁻⁴ M.

Figure 5.

Intensity-dependent open-aperture Z-scans of $C_{60}(>>DPAF-EG_{12}C_1)$ -derived bilayer nanovesicle sample prepared at the concentration of (a) THF-DMSO- H_2O (1:1:38) solvent reference, (b) 5.0×10^{-5} , (c) 1.0×10^{-4} , and (d) 5.0×10^{-4} M. Measurements (symbols) were carried out with a 500-fs laser duration at 780 nm. The solid curves are the best fits with Eqn. (2).

Figure 6. Concentration-dependent saturation intensity (I_s) and two-photon absorption coefficient (β) values of $C_{60}(>DPAF-EG_{12}C_1)$ -derived bilayer nanovesicle solutions.

Figure 7. Concentration-dependent two-photon absorption cross-section values of $C_{60}(>DPAF-EG_{12}C_1)$ -derived bilayer vesicle solutions.

Figure 8.

Nonlinear light-transmittance of the solvent reference [THF–DMSO–H₂O (1:1:38)] and C₆₀(>DPAF-EG₁₂C₁)-derived bilayer nanovesicle samples prepared at various concentration, as indicated. (Curves were fitted with Eqn. (2)).

Scheme 1.

Synthesis of amphiphilic C_{60} -fluorene *antenna* derivative $\text{C}_{60}(>\text{DPAF-EG}_{12}\text{C}_1), \mathbf{4}$.

Reagents and synthetic conditions: (i) $(\text{CH}_3)_3\text{Si-I}$, CHCl_3 , 60°C ; (ii) PEG-bis(carboxymethyl) ether, DCC (2.2 equiv.), DMAP, $\text{ClCH}_2\text{CH}_2\text{Cl}$; (iii) MeOH.