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Water-soluble inclusion complex of fullerene with γ-cyclodextrin polymer for photodynamic therapy

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Abstract

A stable aqueous inclusion complex of fullerene (C60) with macromolecules (C60 concentration as high as 3×10^{-4} mol·L^{-1}) was achieved by a one-step strategy using γ-cyclodextrin polymer (γ-CDP). The inclusion complex of C60 with γ-CDP (C60-γ-CDP) was characterized by ultraviolet-visible, Raman, ^{1}H-NMR spectroscopies, powder X-ray diffraction analysis, and thermogravimetric analysis. The supramolecular interaction and the equilibrium constant for a 1:2 (C60:CD unit in γ-CDP) complex of C60 with γ-CDP were studied. Under ultraviolet A (UVA) irradiation C60-γ-CDP in water could generate singlet oxygen, which was detected by electron paramagnetic resonance spectra. We also evaluated the cytotoxicities of C60-γ-CDP, and investigated the phototoxicity of C60-γ-CDP and pristine C60 toward B16-F10 melanoma cells. The cell viability test showed that C60-γ-CDP had significantly higher photodynamic ability than that of the pristine C60 under UVA

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irradiation. This work demonstrated both a CDP-functionalized strategy for enhancing the water-solubility and phototoxicity of fullerenes for applications to cancer photodynamic therapy, as well as a remediation for the negative biological effects of pristine fullerenes.

1. Introduction

Cyclodextrins (CDs) are commercially available cyclic oligosaccharides, constituted of 6, 7 or 8 glucose units linked by α-1,4-glucosidic bonds.[1] The most commonly used CD has an internal cavity diameter varying among 5, 7 and 9 Å for α-, β- or γ-CD, respectively. Given the hydrophobic internal cavity and hydrophilic external surface of CDs,[2] CDs have frequently been applied in many fields, such as electrochemistry,[3, 4] biotechnology,[5-7] and environmental protection,[8, 9] because their enchanting molecular structures can form supramolecular host-guest complexes with various hydrophobic molecules. CD Polymer (CDP) with the complex forming properties of CD and high solubility, so it can either partially or entirely accommodate suitably sized hydrophobic molecules[10, 11] or nanomaterials,[12] and form the water-soluble host-guest inclusion complexes with hydrophobic and van der Waals interactions. Moreover, it has gained increasing attention and been widely exploited for biomedical science in the recent years.[13-15]

Currently, various applications of Fullerenes (C60s) have been rapidly increased in wide industrial fields and biomedicines due to their unique electronic properties and biological activities.[16] Specifically, C60, as a carbonaceous nanomaterial, can be

photo-chemically activated under photo-irradiation to produce singlet oxygen ($^1O_2$) with high quantum efficiency.[17-19] The process can make effective sensitized oxidation of organic pollutants and inactivation of cells with relatively low energy input.[20] However, the potential application of fullerene as a biochemical photocatalyst in water treatment is limited owing to the hydrophobic surface of C60. In spite of these unique photochemical, electrochemical, and mechanical properties of C60, its extremely poor water-solubility has significantly impeded medicinal applications.[21-23] Therefore, the method to disperse C60 in water has been one of the hot topics for the past few years. Similarly, much effort has been focused on the increase of C60 water-solubility by designing several water-soluble fullerene derivatives approaches.[24-26] However, the chemical modifications usually restrict C60 photo-physical properties.[27, 28] Therefore, solubilization of C60 with non-covalent approaches is good for photochemical applications of C60. CD, as a suitable solubilizing agent, can provide hydrophobic cavities in aqueous solutions for C60 to form inclusion complexes because of their suited cavity size.[29] Furthermore, the formation of C60 inclusion complexes with CD can significantly reduce C60 aggregation, preserving the photosensitizing ability of C60.

In a previous study, we reported the formation of stable inclusion complex of C60 with β-CDP (C60-β-CDP).[12] As we known, C60-CD complexes were widely used in biomedical applications,[30] and there was no report about the degradation of CD in the presence of $^1O_2$. Because the reactions of $^1O_2$ often involved carbon-carbon double bond, such as Alder-ene reaction, and Diels-Alder reaction.[31] However, CD in

water did not have the chemical structure which could react with $^1O_2$. It was meant that C60-CD complexes could be stable when the generation of $^1O_2$, which provided advantageous conditions for its aqueous application. Here, γ-CDP was chosen as the host polymer because of its high water-solubility and right cavity size for C60. The supramolecular interaction between γ-CDP and C60 was firstly discussed. We also evaluated the ability of C60 inclusion complex with γ-CDP (C60-γ-CDP) to generate $^1O_2$ after ultraviolet A (UVA) irradiation, and determined the excellent photodynamic activity of C60-γ-CDP against cancer cells. The result reserved that CDP-functionalized methodology of fullerenes without any chemical modification was advantageous to investigate the structure–performance relationship between fullerenes with supramolecular chemistry to design compounds for special applications.

2. Experimental

2.1. Reagents

Fullerene (C60), γ-cyclodextrin (γ-CD), epichlorohydrin (EP), ethylene glycol, and 2,2,6,6-tetramethyl-4-piperidone (TEMP) were purchased was purchased from Sigma-Aldrich. Double distilled and sterilized water was used to prepare all solution.

RPMI 1640 medium and fetal bovine serum were purchased from Thermo Scientific. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertazolium bromide (MTT) was purchased from Sigma Chemical Co. Penicillin and streptomycin were from Beyotime Institute of Biotechnology.

2.2. Apparatus

The molecular-weight distribution of γ-CD polymer was determined by gel permeation chromatography (GPC) Agilent 1100 series (Agilent, USA) with PLaquagel-OH MIXED 8 μm column. Ultraviolet-visible (UV) spectra were recorded on the UV spectrum photometer (Shimazu, UV-2550) equipped with a quartz cell (1.0 cm optical path length). The ^{1}H NMR spectra were conducted on a 600 MHz NMR spectrometer (Bruker, AVANCE 600) at 303.1 K in deuterium oxide. The powder X-ray diffraction spectra (XRD) were measured by a X-ray instrument (Bruker, D8 super speed) with Cu Kα radiation, λ=1.542 Å. The Raman measurements were carried out on a Raman system (Renishaw, Renishaw inVia). Thermo gravimetric analysis (TGA) was performed on a thermogravimetric analyzer (PerkinElmer, Pyris 1 TGA) with 10 mg samples which was heated from room temperature to 700 ℃ at a rate of 10 ℃·min^{-1} under nitrogen atmosphere. Electron Paramagnetic Resonance (EPR) Spectra were carried out with a EPR spectrometer (Bruker., A300-10/12) under the following conditions: 10 mW microwave power, 100 kHz modulation frequency, 1 G modulation amplitude, scan time 8 min, and 80 G scan range. The microscopic observation of the preliminary cell viability assay was used by a light microscopy (Nikon, 80i). The optical density of each well was measured at 570 nm using a microplate reader (Bio-TEK, elx800).

2.3. Preparation of γ-CD polymer and C60-γ-CD polymer inclusion complex

The water-soluble γ-CDP was obtained by polymerization of γ-CD with EP under a strongly alkaline condition (30 wt% NaOH), which were close to the ones described the methods of preparation of water-soluble CD polymer.[10, 32, 33]

The synthesis and purification of C60-γ-CDP were shown as follow: it was prepared by dissolving 4 g γ-CDP and 2 g C60 in 100 ml water with sufficiently stirring for at least 48 h at room temperature. At the end of the reaction, a brown solution contained C60-γ-CDP was obtained after filtrating to remove insoluble C60. After that, 200 ml ethanol was added into the solution. The inclusion complex precipitated from the solution. Then C60-γ-CDP was isolated by filtration using a membrane filter (pore size: 0.3 μm), and washed by ethanol. The inclusion complex was dried in a vacuum oven at 60 ℃ for 24 h. The schematic illustration of γ-CDP, C60, and C60-γ-CDP was shown in Fig. 1.

2.4. Singlet Oxygen Detection by Electron Paramagnetic Resonance

Singlet oxygen was detected by an EPR method using TEMP as a spin-trapping reagent. To 5 mL C60-γ-CDP and TEMP aqueous solution (C60 in C60-γ-CDP: 80 μM, TEMP: 40 mM) was introduced into a flat cell, irradiated with a 300 W photo-reflector lamp at a distance of 10 cm, and immediately subjected to EPR measurement. The generation of $^1O_2$ was detected as an EPR signal due to TEMPO formed by the reaction of $^1O_2$ with TEMP.[18] Radiation from the lamp was passed through a glass filter to remove wavelengths below 300 nm.

2.5. Cell culture

The mouse melanoma cell lines B16−F10 were purchased from the Cell Bank of the Chinese Academic of Sciences. The mouse melanoma B16−F10 was maintained at 37 ℃ at 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin and streptomycin.

2.6. Cell Viability

The photocytotoxicity test was described in the previous study with minor modifications.[34, 35] Briefly, B16−F10 cells were plated at a density of 4×10^5 cells ml^{-1} in 96-well plates in RPMI 1640 medium supplemented with 10% fetal bovine serum for 1 h. After that, the medium was removed and replaced by sterile PBS. The cells were cultured in dark for 2 h at 37 ℃ to different C60-γ-CDP in phosphate buffer solution (PBS). Also, control cells were treated with PBS alone. Cells were then irradiated with UVA from two fluorescent PUVA lamps (Philips, PL-L36W) or two cool white visible light lamps (Philips，TLD36W). After 20 minutes of exposure, the PBS solution was removed and replaced with cell culture medium, and the cells were kept in the incubator overnight. 20 μl of MTT (5 $mg\cdot ml^{-1}$) was added to each well, and the cells were further incubated for an additional 4 h. After incubation, media was removed and DMSO was added to dissolve purple precipitates. Then plates were read at 570 nm using a microplate reader (Bio Tek, EXL800).

2.7. Statistical analysis

Statistical analyses were conducted using SPSS ver 11.5. Results are expressed as mean ± SEM. and the significance of differences was determined using the two-way analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple comparison test (SNK) as post hoc. Differences were consider significant if $P<0.05$.

3. Results and discussion

3.1. Aqueous solubility and dissociated constant of C60-γ-CDP

C60 is essentially insoluble in water. It was observed that C60 (10 mg) did not dissolve in water (10 mL) even upon stirring at 25 ℃ for 24 h, as shown in Fig. 2A (a). However, C60 suspension was obtained when 100 mg of CDP was used and stirred at 25 ℃ for 24 h by a magnetic stirrer. The suspension was centrifuged in order to separate the undissolved C60 precipitates. After filtration, a brown filtrate was obtained in Fig. 2A (b). This result showed γ-CDP as the solubilizing agent made C60 dispersing in water, indicating that γ-CDP, as well as γ-CD[36] and β-CD polymer,[12] could form inclusion complexes with C60.

Fig. 2B shows the UV absorption spectra of C60 (a) and C60-γ-CDP (b) in water. Because of the poor water-solubility of C60, no absorption of C60 was observed in the range of 200-800 nm as shown in Fig. 2B (a). However, the UV spectrum of C60-γ-CDP showed a characteristic absorption band at 220, 272, and 333 nm corresponding to the chromophoric C60 molecules in Fig. 2B (b), thereby proving that the C60 molecules were dissolved in water and γ-CDP served as the solubilizing agent. Compared with the UV spectrum of C60-γ-CD in water,[37] the three absorption bands

in Fig. 2B (b) red-shifted, indicating that C60s could form inclusion complexes with γ-CDP.

Fig. 3A shows the UV spectra of C60-γ-CDP with different concentrations in aqueous solution. The peak positions were independent of the concentrations of C60-γ-CDP, suggested that C60 could not form C60 aggregation in water with γ-CDP. It was meant that the supramolecular interaction prevented C60 from forming the aggregation in water. Moreover, the peaks intensity increased with the concentration of C60-γ-CDP. Plotting the absorbance of C60-γ-CDP at 272 nm versus the concentration of C60-γ-CDP, a straight line was obtained as shown in Fig. 3B. According to the Lambert-Beer law, the absorption coefficient (ε) of C60-γ-CDP in aqueous solution (pH=7.0, 25℃) were evaluated as 1.31 $L \cdot g^{-1} \cdot cm^{-1}$.

In order to study the supramolecular interaction between C60 and γ-CDP, the Benesi-Hildebrand method was used by the UV spectrum.[38] Because of the insolubility of C60 in water, ethylene glycol, as a suitable solvent for C60 and γ-CDP, was chosen to study the dissociated constant of the inclusion complex. The formation of C60-γ-CDP in ethylene glycol could be confirmed by the UV spectrum as shown in Fig. 4A, where the concentration of CD unit in γ-CDP was varied from 1×10^{-6} to 3.6×10^{-5} $mol \cdot L^{-1}$ (C60 concentration: 6.94×10^{-5} $mol \cdot L^{-1}$). The peak position was also independent of the addition of γ-CDP, however, the peak intensity increased with γ-CDP. Assuming the mole ratio of C60 to CD unit in γ-CDP was 1:2, the formation of the inclusion complex could be calculated as follows:[11]

$$\frac{[H]_0^2[G]_0}{\Delta A} = \frac{[H]_0^2}{\Delta \varepsilon} + \frac{K_D}{\Delta \varepsilon} \quad (1)$$

where H represented the host, CD unit in the polymer, G was the guest, C60, and the initial concentrations of H and G were $[H]_0$ and $[G]_0$ respectively, and $[H]_0 \gg [G]_0$, K_D was the dissociation constant, ΔA was the change in the measured absorbance, and $\Delta\varepsilon$ was the change in the molar absorption coefficients.

Plotting $[H]_0^2[G]_0/\Delta A$ versus $[H]_0^2$, a straight line was obtained in Fig. 4B. The good linear relationship proved 1:2 ratio of C60 to CD unit in γ-CDP. According to the slope and the intercept of the line, K_D of the inclusion complex was evaluated as 6.36×10^{-5} mol·L^{-1}. The K_D of C60-γ-CDP was smaller than that of C60-γ-CD (8.20×10^{-5} mol·L^{-1}, which was the reciprocal value of the formation constant, 1.22×10^{4} L·mol^{-1} [39]), suggesting that γ-CDP could form the inclusion complex more easily than γ-CD.

According to the 1:2 mole ratio of C60 to CD unit in γ-CDP, the water solubility of C60 in C60-γ-CDP was calculated to be 3.0×10^{-4} mol·L^{-1}. The aqueous solubilities of C60 in different supramolecular inclusion complex[12, 39-42] are presented in Table 1. It was found that γ-CDP made the aqueous solubility of C60 higher than γ-CD, γ-CD thioether, 6-amino-γ-CD and β-CDP because of its high water solubilities. And it was clear that the solubility of C60 in C60-γ-CDP was about 2×10^{8} times greater than that of C60.[43] These results revealed that the water-soluble host γ-CDP improved the aqueous solubility of C60 remarkably by the formation of C60-γ-CDP inclusion complex.

In short this supramolecular method using γ-CDP had the following three advantages: (i) not only C60 but also other fullerene derivatives with hydrophobic or

hydrophilic functional groups could be dispersed; (ii) C60-γ-CDP aqueous solutions with high concentration were stable; and (iii) it could decrease the aggregation of C60, which was good for pharmaceutical applications.

3.2. Characterization of C60-γ-CDP

The number average molecular weight (M_n) of γ-CDP was measured as 55000 g·mol^{-1} by GPC. The typical ^{1}H NMR spectrum of γ-CDP was presented in the Electronic Supplementary Information (ESI, Fig. S1 (a)), which was consistent with the previous report.[32] Although the chemical shift of C60 was not showed in the ^{1}H NMR spectra of C60-γ-CDP (Fig. S1 (b)), the upfield shifts of CD unit (H1-H6) in C60-γ-CDP distinguished for the ^{1}H NMR spectra strongly confirmed that C60 monomers entered into the hydrophobic CD cavities of γ-CDP, resulting in the change of γ-CDP micro-environment. The result was also similar to our previous studies in CDP inclusion complexes,[10-12] suggested formation of C60-γ-CDP by supramolecular interaction.

The inclusion complex was also confirmed by X-ray diffractometry.[10, 44] Fig. 5 shows the X-ray diffraction pattern of (a) C60 and (b) C60-γ-CDP. In Fig. 5 (a), the sharp peaks of C60 at diffraction angles of 2θ 10.8°, 17.7°, 20.8° were observed, showing that C60 existed as a crystalline material. The X-ray diffraction pattern of the C60 inclusion complex in Fig. 5 (b) shows that typical peaks of C60 Peak intensity decreased, a broad peak appeared at 2θ=18.8°, and the peaks above 21° disappeared. The complex had a different structure to the parent γ-CDP and C60, indicating that

C60-γ-CDP had a new crystalline phase associated with the formation of C60-γ-CDP. The result was in accord with similar observations for the γ-CD complex.[37]

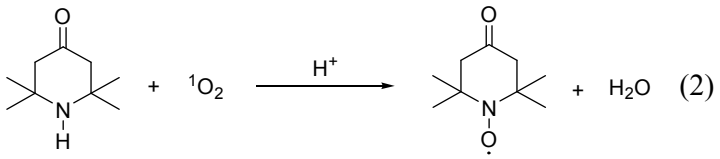
The interaction between γ-CDP and C60 could also be studied from the Raman spectra. The Raman spectrum of γ-CDP was obtained in Fig. 6 (a), and no active Raman was found in the range of the wavenumbers 1200–1800 cm^{-1}. Fig. 6 (b) and (c) shows the Raman spectra of C60 and C60-γ-CDP. The Raman dominant peak of C60-γ-CDP at 1470 cm^{-1} represented the stretching mode of cages of C60, which slightly shifted (down to 4 cm^{-1}) compared with that of C60 (1464 cm^{-1}) which was similar to C60-β-CDP,[12] and suggested that the formation supramolecular complexes did not change the nature of C60.

The thermal stability of the inclusion complex was determined by TGA in a temperature range of 25-700 ℃. Fig. 7 shows the TG curves of the inclusion complexes of (a) C60 and (b) C60-γ-CDP. In Fig. 7 (a) a loss of weight in the 250 ℃ to 400 ℃ temperature range corresponding to the thermal decomposition of γ-CDP. In Fig. 7 (b), the mass loss (250-400 ℃) corresponding to the decomposition of γ-CDP in the inclusion complex, and C60 was thermally stable. Thus, the amount of C60 was determined to be 18.5 wt% for C60-γ-CDP, and the molar ratio of C60 and γ-CD unit γ-CDP was calculated as 1/1.9 which was close to the above study.

3.3. Detection of Singlet Oxygen Generation

Usually, $^1O_2$ was detected by the $^1O_2$ quencher or the direct $^1O_2$ phosphorescence method. For some researches of C60-CD inclusion complexes,[34, 35, 45, 46] the $^1O_2$

generation ability of C60-CD was studied by the EPR method. In order to compare the $^1O_2$ generation effects between C60-γ-CDP and C60-CD, we chose the EPR method. It was reported that EPR spectra could study $^1O_2$ by detecting a nitroxide radical,[18] 2,2,6,6-tetramethyl-4-piperidone-N-oxyl radical (TEMPO), which was generated from TEMP and $^1O_2$ (2).



(2)

Fig. S2 in ESI shows the three typical signals of TEMPO, suggesting that TEMP reacted with $^1O_2$ to give a $^1O_2$ adduct, TEMPO, and the relative intensity of the TEMPO increased with the photoirradiation time. It was meant that C60-γ-CDP solutions could also produce $^1O_2$ by UVA photo-irradiation as well as C60/β-CD and $(\gamma\text{-CD})_2$/C60.[35,46]

Fig. 8 shows the EPR intensity of the TEMPO signal as a function of time of irradiation for C60-γ-CDP solutions with UVA or visible light. The EPR spectra of C60-γ-CDP showed great rate of $^1O_2$ production as measured by an increased TEMPO signal with UVA irradiation. It was reported that C60 aggregation could deactivated $^1O_2$ quenching.[35, 47] However, Our result showed that the microenvironment of C60 in γ-CDP facilitated the generation of $^1O_2$, because C60 molecules with high concentration dispersed well in water by formation of γ-CDP inclusion complex. Therefore, C60-γ-CDP had a high potential for generating $^1O_2$ with photo-irradiation. Furthermore, with visible light irradiation, the rate of $^1O_2$ production was very low and hardly increased with the irradiation time. The result was different with

C60/β-CD[46] that C60-γ-CDP could only generate $^1O_2$ with UVA irradiation, indicating the possibility of the controllable $^1O_2$ production in photodynamic therapy.

3.4 The effects of γ-CDP and C60-γ-CDP on cell viability in B16-F10 cells

The biotoxicities of γ-CDP and C60-γ-CDP were very important for the photodynamic therapy. To determine the cytotoxic potential of γ-CDP and C60-γ-CDP, B16-F10 cells were incubated with various concentrations of γ-CDP or C60-γ-CDP for 24 and 48 h and cell viability was evaluated by MTT assay. Fig. 9 A and B show that no cytotoxicity was observed when the C60-γ-CDP complexes were added to the cells in the absence of any exposure to light for 24 and 48 h at concentrations from 0.5 μM to 20 μM. The similar results showed in Fig. 9 C and D, that γ-CDP did not affect cell viability for 24 and 48 h, indicating γ-CDP could not have a negative impact on the photodynamic therapy and be potential for pharmaceutical carriers. Moreover, the cellular morphological photos of B16-F17 cells were microscopically observed after treatment with C60-γ-CDP or γ-CDP (1-20 μM) for 48h in ESI. Fig. S3 shows that the density of melanoma cells was almost not reduced by the treatment of C60-γ-CDP, and the morphological photos of the cells with γ-CDP (Fig. S4) were similar to Fig. S3. These result proved that γ-CDP as a host molecule could not only increase the apparent solubility of the guest, but also improve the bioavailability of C60.

3.5 Phototoxicity to B16-F10 Cells

For these years, the chemotherapeutic strategies have showed a little effect

against metastatic melanoma.[48] Photodynamic therapy of C60-γ-CDP, as a potential new approach for treatment of dermal melanoma, was studied when the tumor (B16-F10 cells) was irradiated with UVA. To determine the phototoxicity of C60-γ-CDP and C60 on cell viability, B16-F10 cells were initially seeded in microplates followed by different treatments. The results of the MTT assay indicated that no obvious effect on viability was observed when B16-F10 cells were exposed to C60-γ-CDP or C60 in the dark or in the presence of visible light (Fig. 10). With UVA irradiation, however, C60-γ-CDP caused a dramatically reduced rate of cell viability as the increase of C60-γ-CDP. These results were consistent with the above EPR spectra, and they also suggested that $^1O_2$, which produced by C60-γ-CDP photo-irradiation, induced the efficient damage of B16-F10 cells. Notably, the concentration of C60-γ-CDP which was used to kill B16-F10 cells could be as low as 0.5 μM, and the phototoxicity of C60-γ-CDP was about 40 times higher than C60.

Because of the high water-soluble γ-CDP and unique electronic π-system of C60, C60-γ-CDP could be dispersed in aqueous solution by formation of CDP inclusion complex and generate $^1O_2$ upon irradiation with UVA through the energy and electron transfer processes.[49, 50] The precious results confirmed that C60 inclusion complexes with CD in water were present in C60 aggregation.[35, 51] As we known, the state of C60 aggregation could deactivate the excited electronic states of photo-sensitizers and cause further loss of photo-reactivity, and monomeric C60 were more phototoxic than aggregates of C60.[34, 35, 50] In this inclusion complex, C60 molecules could penetrate into CD cavities in γ-CDP and prevent to form C60 aggregation. This would explain

the observed difference in phototoxicity between C60-γ-CDP and C60, as shown in Fig. 10A.

Although C60-CD complexes was not more efficient in photodynamic therapy than C60 derivative[22] due to the restriction of CD cavity,[39] the supramolecular method by γ-CDP improved C60 water-solubility and biocompatibility practically and provided an easier way to large-scale syntheses of water-soluble fullerene than chemically derivatized method of C60. It was clear that the use of CDP opened opportunities for designing highly versatile inclusion complexes with often improved properties of guest molecules compared to conventional non-CD systems. Because of their fascinating properties, it was expected that CDP based C60 systems would find their way to clinical applications.

4. Conclusions

In summary, we developed a simple and fast method to obtain a high water-soluble C60-γ-CDP inclusion complex. The supramolecular interactions between host and guest molecules significantly preserved the integrity of C60, which was critical for many applications. C60-γ-CDP could efficiently generated $^1O_2$ species with UVA irradiation, and be regarded as a safe inclusion complex due to the low cell toxicity without UVA irradiation. And γ-CDP not only imparted solubility to the hydrophobic C60 in aqueous solution with less aggregation, but also increased biocompatibility efficiently. In addition, C60-γ-CDP with high water solubility and singlet oxygen generation ability showed great phototoxicity for B17-F10 melanoma

cells. Furthermore, we believed that the CDP-functionalized methodology would lead to better investigation of the fullerene structure–performance relationship and biomedical applications.

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446 **Table 1** Aqueous solubility of C60 in different supramolecular inclusion complex

Sample	Concentration of C60 in aqueous solubility ($mol \cdot L^{-1}$)	Reference
C60-γ-CDP	$\mathbf{3.0\times10^{-4}}$	our work
C60/γ-CD	1.0×10^{-4}	39
C60/γ-CD thioether	2.1×10^{-5}	40
C60/6-amino-γ-CD	1.0×10^{-5}	41
C60-β-CDP	6.7×10^{-5}	12
C60 cluster	1.0×10^{-5}	42
C60	$<1.4\times10^{-12}$	43

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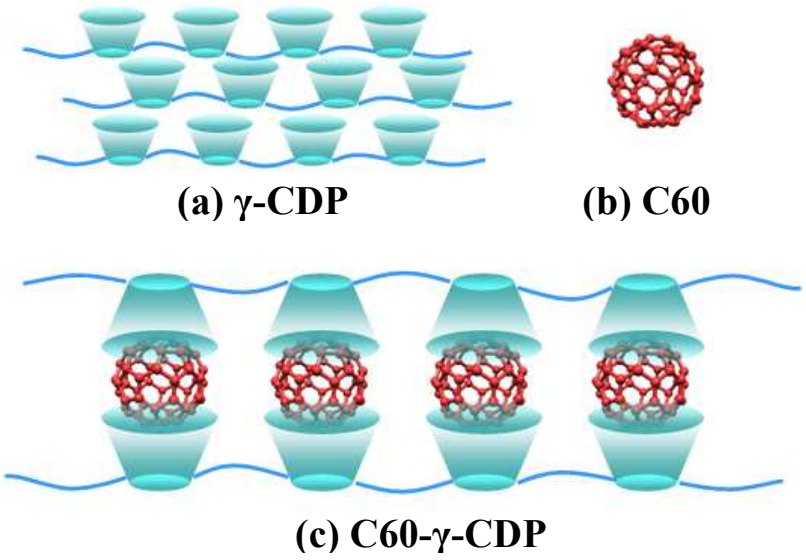
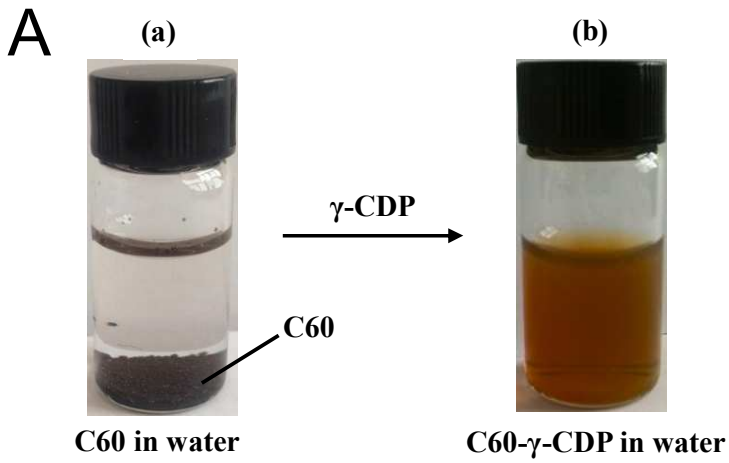


Fig. 1 Schematic illustration of (a) γ-CDP, (b) C60, and (c) C60-γ-CDP



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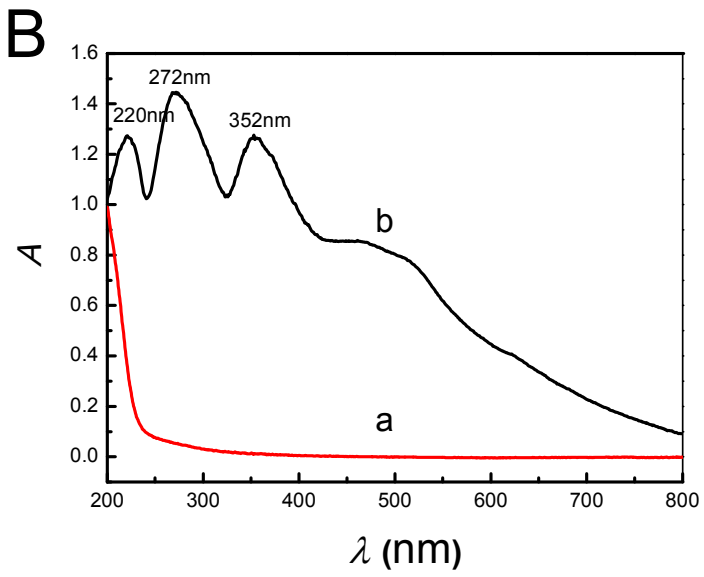

454

455 **Fig. 2A** Photographs of (a) C60 and (b) C60-γ-CDP in water. **B** UV spectra of (a) C60

456 and (b) C60-γ-CDP in aqueous solution pH=7.0, at 25℃.

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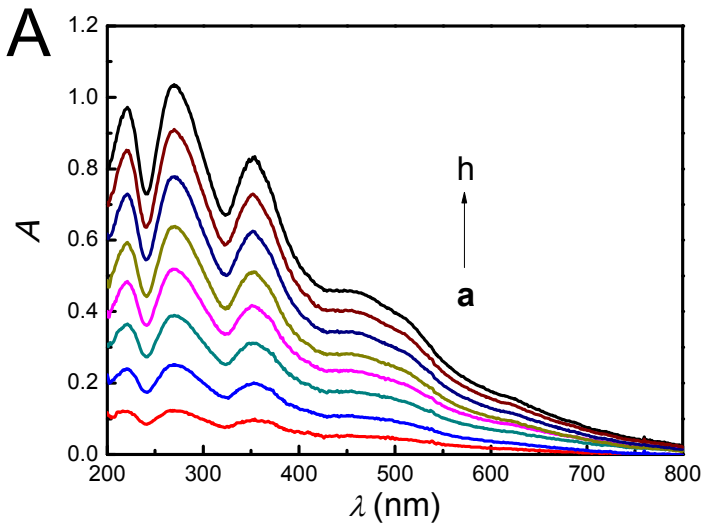

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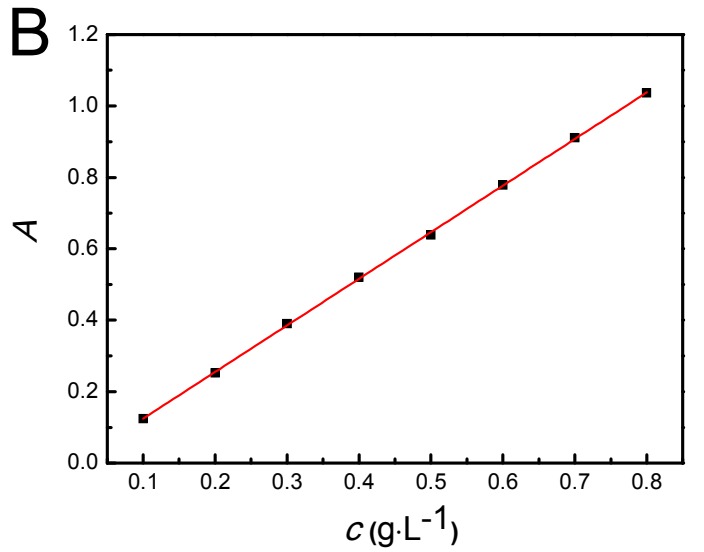
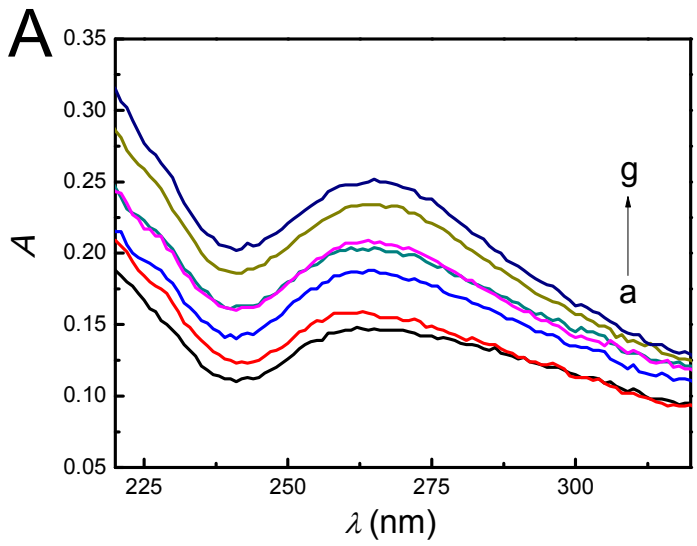


459

460 **Fig. 3A** UV spectra of C60-γ-CDP with different concentrations (g L^{-1}) in aqueous
461 solution (pH=7.0, 25℃): (a) 0.1, (b) 0.2, (c) 0.3, (d) 0.4, (e) 0.5, (f) 0.6, (g) 0.7, and (h)
462 0.8. **B** A plot of absorbance ratio of C60-γ-CDP at 272 nm vs. the concentration of
463 C60-γ-CDP, Date taken from **Fig. 3A**.



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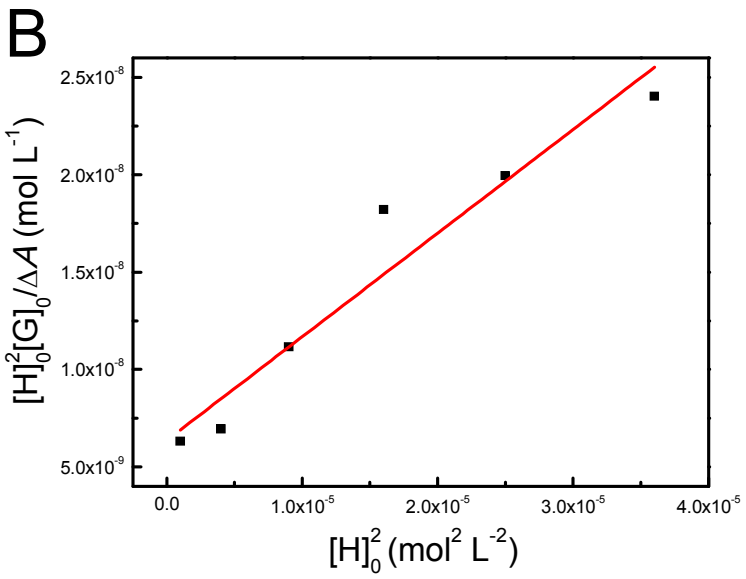

465

466 **Fig. 4A** At 25 ℃, UV spectra of 6.94×10^{-5} mol·L^{-1} C60 in ethylene glycol with

467 different concentrations of γ-CD unit in CDP (mol·L^{-1}): (a) 0, (b) 1×10^{-6}, (c) 4×10^{-6}, (d)

468 9×10^{-6}, (e) 1.6×10^{-5}, (f) 2.5×10^{-5}, (g) 3.6×10^{-5}. **B** The plot of $[H]_0^2[G]_0/\Delta A$ vs. $[H]_0^2$.

469 Data taken from **Fig. 4A**.

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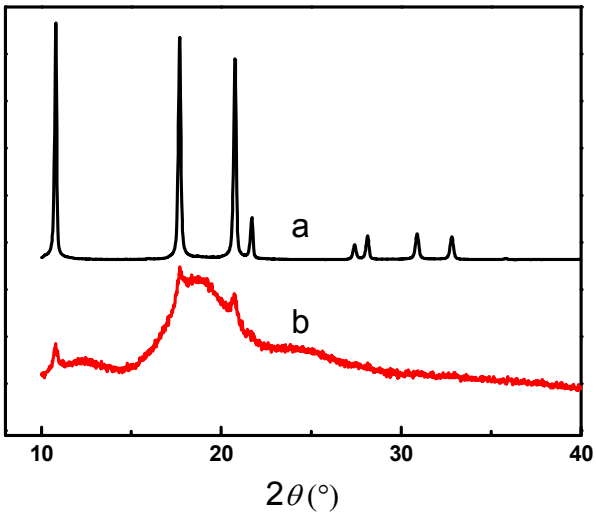

471

472 **Fig. 5** Powder X-ray diffraction patterns of (a) C60 and (b) C60-γ-CDP.

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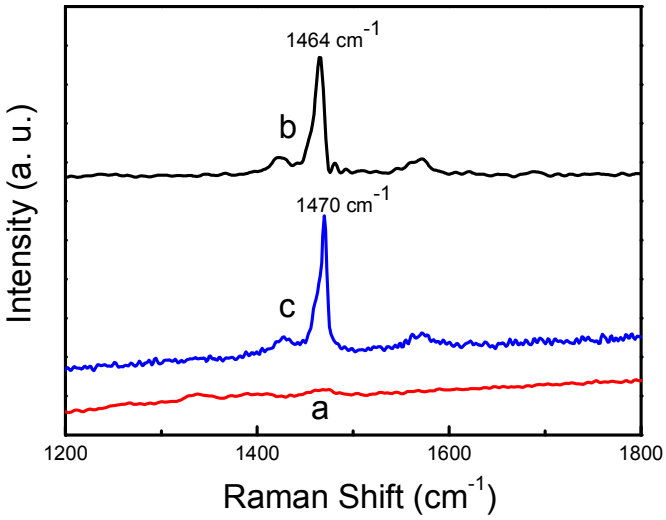


475

476 **Fig.6** Raman spectra of (a) γ-CDP, (b) C60, and (c) C60-γ-CDP.

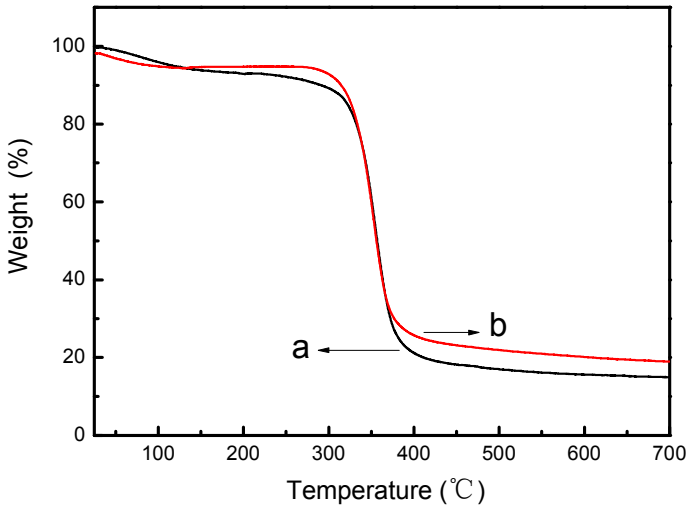


Fig. 7 TG curves of (a) γ-CDP and (b) C60-γ-CDP.

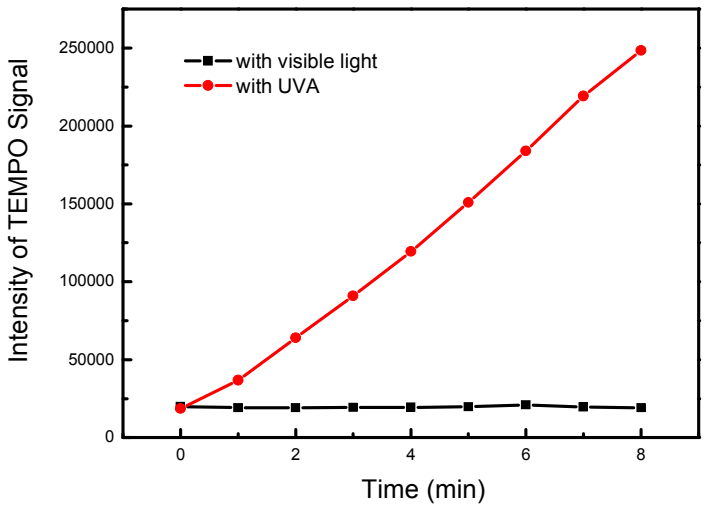
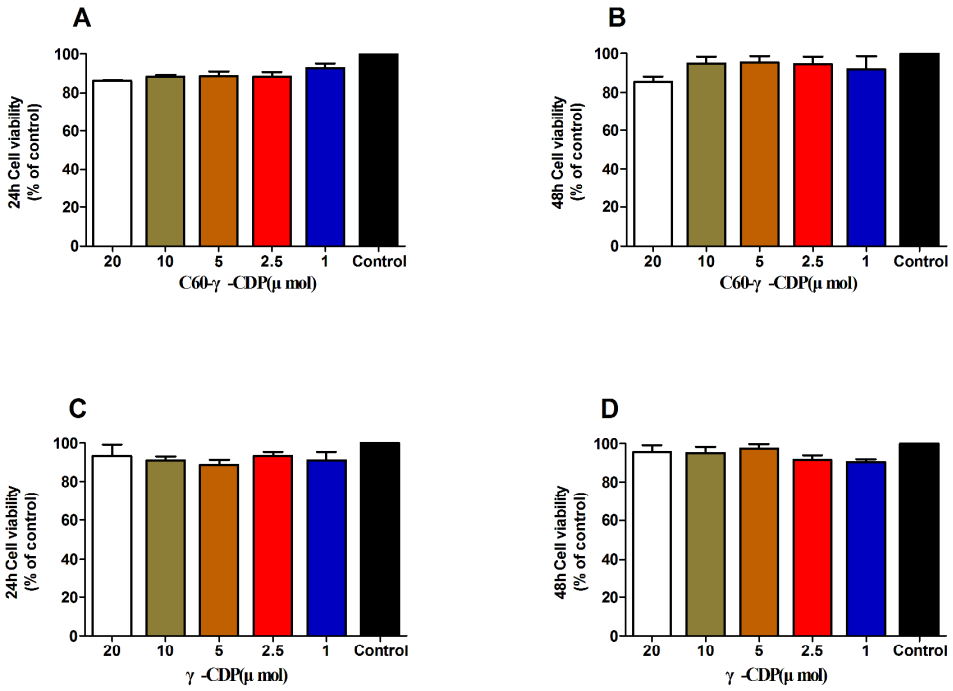


Fig.8 Intensity of TEMPO signals as a function of time during UVA or visible light irradiation of C60-γ-CDP water solution.



485

486 **Fig. 9** *In vitro* cytotoxicity of 1-20 μM C60-γ-CDP (A 24h and B 48h) and γ-CDP (C
487 24h and D 48h) following the MTT assay.
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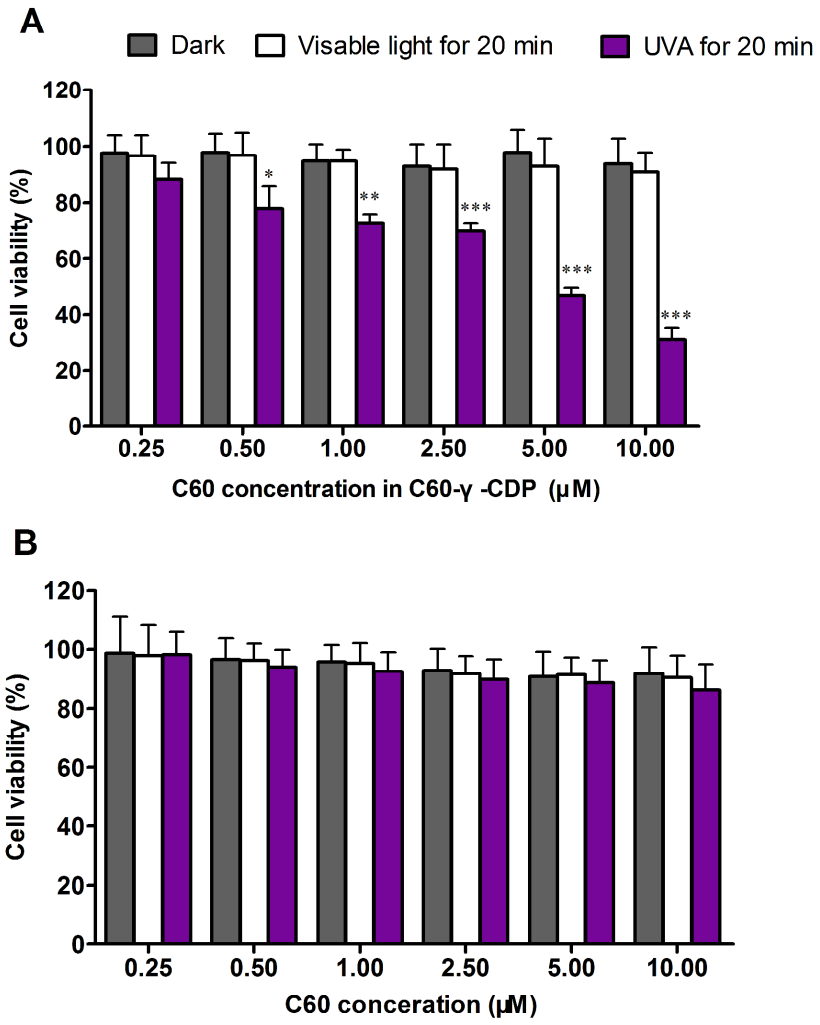

496

497 **Fig. 10** Effect of different concentrations of (A) C60-γ-CDP and (B) C60 exposure on

498 the viability of B16-F10 cells irradiated with UVA and cool white light as measured

499 by the MTT assay (see the Materials and Methods).

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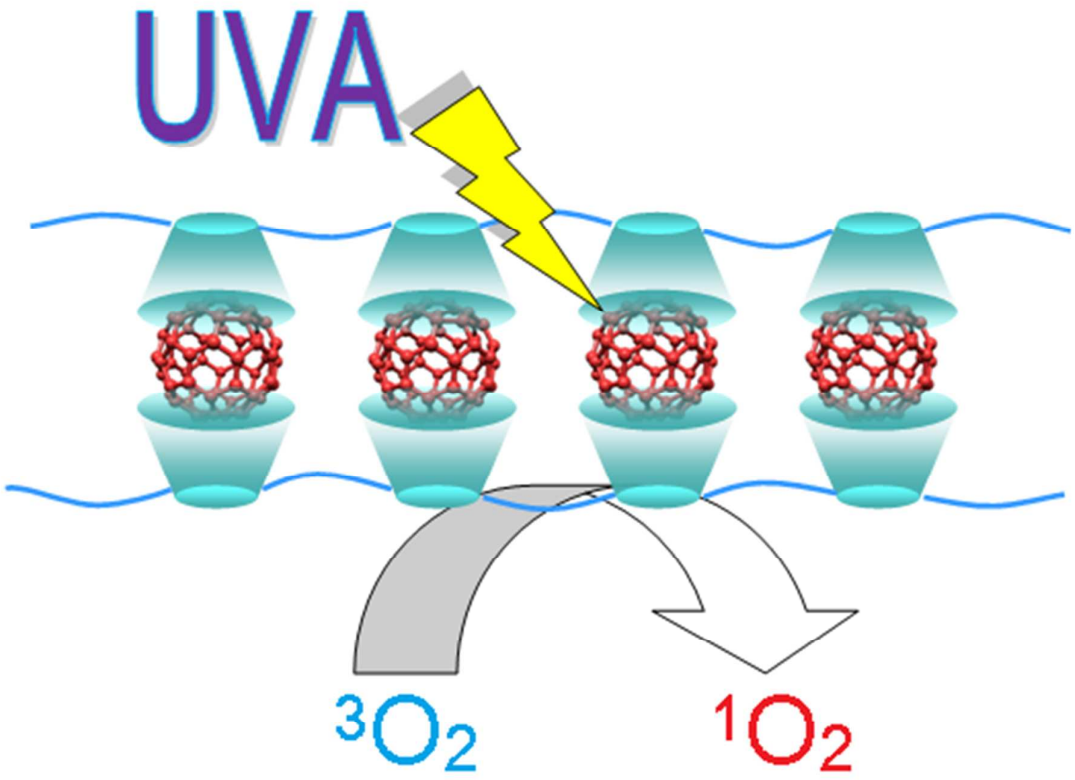
A table of contents entry



Text:

A method was developed to obtain a high water-soluble C60-γ-CDP inclusion complex, which could efficiently generated $^1O_2$ species with UVA irradiation.