Electronic Supplementary Material (ESI) for Chemical Communications

Solubilization of Pristine Fullerene by the Unfolding Mechanism of Bovine Serum Albumin for Cytotoxic Application

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Experimental Details

Chemicals and Reagents. Bovine serum albumin (BSA, MW 67000), fullerene-C_{60} (C_{60}, purity: 99.9%), and Sephadex G-100 were obtained from Sigma (St Louis, MO, USA) and used without further purification. Mouse embryonic fibroblast cell line (BALB/3T3) and human glioma cell line (U87) were obtained from American Type Culture Collection (ATCC). All other chemicals were of analytical reagent grade and used as received. Doubly distilled water was used throughout and aqueous solutions were stored in the dark at 4 °C.

Solubilization of C_{60} by BSA. C_{60} powder (2.5 mg) was directly dispersed in 5.00 mL 0.2 mM BSA water solution for 12 h at 4 °C. The resulting yellow suspension was centrifuged at 6000 rpm for 6 min and the supernatant was filtered through a 0.45 μm nylon membrane. The obtained filtrate containing BSA solubilized C_{60} (BSA-C_{60}) was stored in dark at 4 °C for the subsequent spectral analysis.

Apparatus and Measurements. UV-vis measurements were performed on a UV-3150 spectrophotometer (Shimadzu, Japan) using 1.0 cm quartz cells. Fluorescence spectra between 290 and 450 nm were recorded on a RF-5301PC spectrophotometer (Shimadzu, Japan) with a 1.0 cm quartz cuvette. The slit width and the excitation wavelength were 5/5 nm and 280 nm, respectively. Circular dichroism (CD) measurements were carried out with a J-810 spectrometer in quartz cells (Tokyo, Japan). The CD spectra of BSA or BSA-C_{60} were recorded over a wavelength range of 200–250 nm with a scan speed of 50 nm min^{-1} and a band width of 1.0 nm. Each

CD spectrum was the average of three scans. Transmission electron microscope (TEM) images were taken on a JEM-2010HR transmission electron microscope (JEOL, Japan). TEM grids were prepared by dropping the BSA-C_{60} solution onto 200 mesh carbon coated copper grid and using a blotting paper to remove the extra solution.

Cytotoxicity was measured using mouse embryonic fibroblast cell line (BALB/3T3) and human glioma cell line (U87). The two cell lines were cultured in a High-glucose Dulbecco's Modified Eagle's Medium (H-DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin (100 IU cm^{-3}) at 37 °C in a 5% CO_2 humidified atmosphere, separately. BSA-C_{60} was purified by a column of Sephadex G-100 and freeze-dried by a vacuum. The lyophilized BSA-C_{60} powder was dissolved by 0.01 M phosphate buffered saline (PBS) (pH 7.4) for cytotoxicity tests. The two cell lines (80 μL) were mixed with 20 μL of 0.01 M PBS, 20 μL of 100 μM BSA, or 20 μL of 100 μM BSA containing 120.8 μg mL^{-1} C_{60}, separately, and seeded in 96-well culture plates at a density of 5000 cells/well for BALB/3T3 cells and 4000 cells/well for U87 cells. The plates were pre-incubated for 12 h in dark. After being irradiated for 2 h under visible light (20 mW cm^{-2}) with thermal insulation, the plates were then cultured for 48 h. Controls were prepared by the incubation of the plates for 62 h in dark. The morphology of cells was investigated by microscope. The viability of cells was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as follows: after replacement of the culture medium in each well by a fresh culture medium containing 10 μL of 5 mg mL^{-1} sterile MTT dye (in PBS, pH 7.4), the cells were incubated at 37 °C for 4 h. The MTT solution in each well was

then replaced by 100 μL of DMSO. The optical density (OD) for the formazan product was measured at 490 nm and represented as the percentage of live cells (controls from the incubation of cells mixed with PBS in dark were defined as 100%).

Determination of the dispersion concentration of C_{60}. Based on the literature 1, the indirect UV-vis spectrophotometric method was used to evaluate the dispersion concentration of C_{60}. C_{60} was dispersed in toluene and the absorbance of these dispersions was measured at 335 nm. As shown in Fig. S1, the absorbance increased linearly with the increase in the dispersion concentration of C_{60} from 0.2157 to 9.704 μg mL^{-1}. The linear regression equation was $A_{335} = 0.0881\ c\ (C_{60}, \mu g\ mL^{-1}) + 0.0288$ (R = 0.9997). All the vial, centrifuge tube, syringe filter and syringe that after the solubilization of C_{60} were dried under vacuum for 4 h. The syringe was cleaned by toluene. The filters and the nylon membrane were cut into small pieces carefully. All the pieces were extracted with sonication for 3 times with 20 mL toluene. All the toluene containing C_{60} were adjusted to a volume of 200.00 mL. The absorbance at 335 nm was determined to be 0.598 (Fig. S2), which corresponded to 6.461 μg mL^{-1} C_{60} according to the linear regression equation. Therefore, the residual C_{60} extracted in toluene was 1.292 mg. Because the initial amount of C_{60} was 2.5 mg, the dispersed C_{60} in 5 mL BSA aqueous solution could be calculated to be 1.208 mg. Thus, the dispersion concentration of C_{60} in BSA aqueous solution was 241.6 μg mL^{-1}.

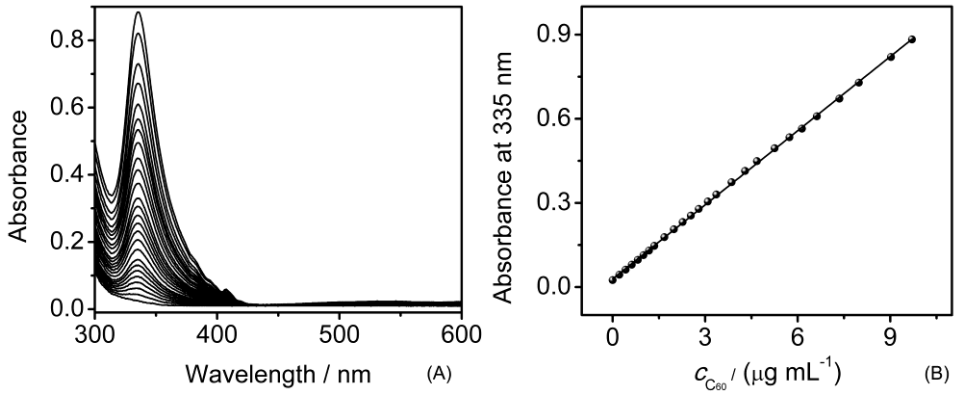

Fig. S1 (A) UV-vis absorption spectra of C_{60} at different concentrations. (B) Dependence of the absorbance at 335 nm with the concentration of C_{60} in toluene. (The concentrations of C_{60} in toluene were from 0.2157 to 9.704 μg mL^{-1}.)

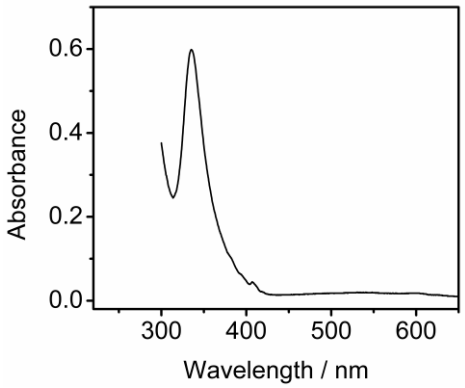

Fig. S2 UV-vis absorption spectra of the initial residual C_{60} extracted in 200.00 mL toluene.

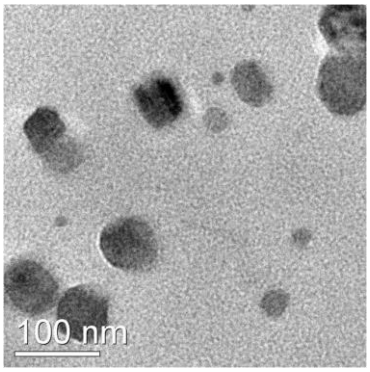

Fig. S3 TEM image of C_{60} dispersed in BSA aqueous solution after 33-day of storage at 4 °C

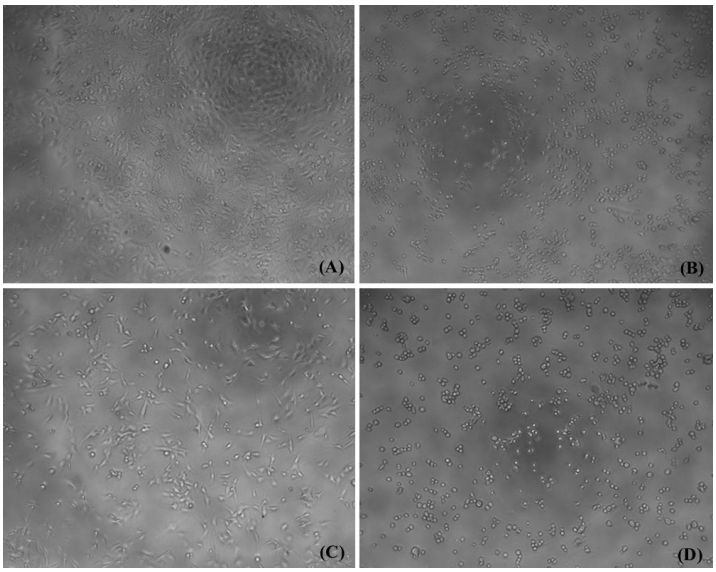

Fig. S4 Phase contrast micrographs of two cell lines cultured for 24 h after visible light irradiation. (A) and (B): BALB3T3 cell lines in the presence of 10 μM BSA containing 12.08 μg mL^{-1} C_{60} and 30 μM BSA containing 36.24 μg mL^{-1} C_{60}, respectively; (C) and (D): U87 cell lines in the presence of 10 μM BSA containing 12.08 μg mL^{-1} C_{60} and 30 μM BSA containing 36.24 μg mL^{-1} C_{60}, respectively

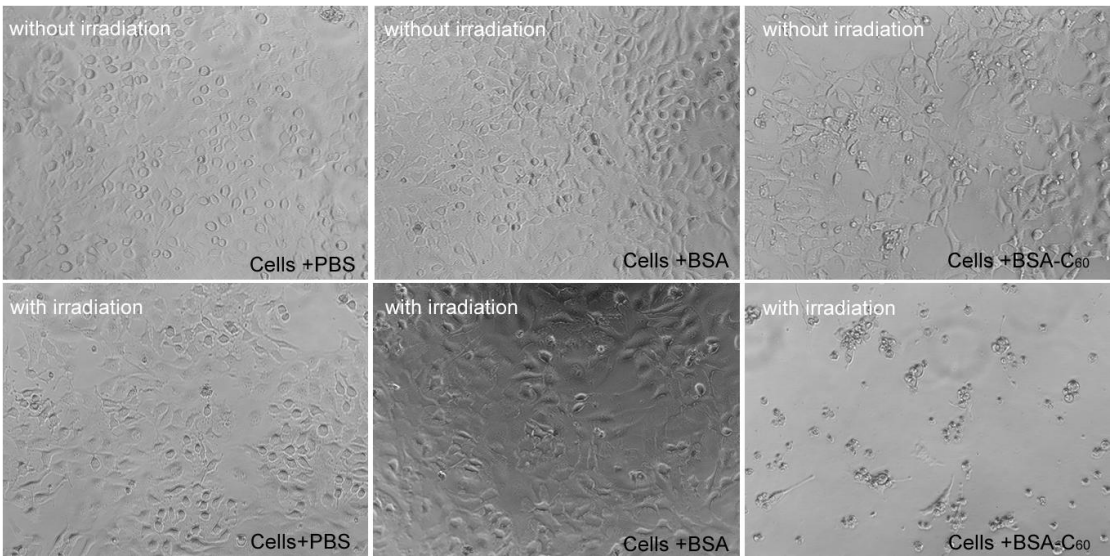

Fig. S5 Phase contrast micrographs of BALB3T3 cell line cultured for 48 h in the presence of PBS, 20 μM BSA, and 20 μM BSA containing 24.16 μg mL^{-1} C_{60} without irradiation and with visible light irradiation.

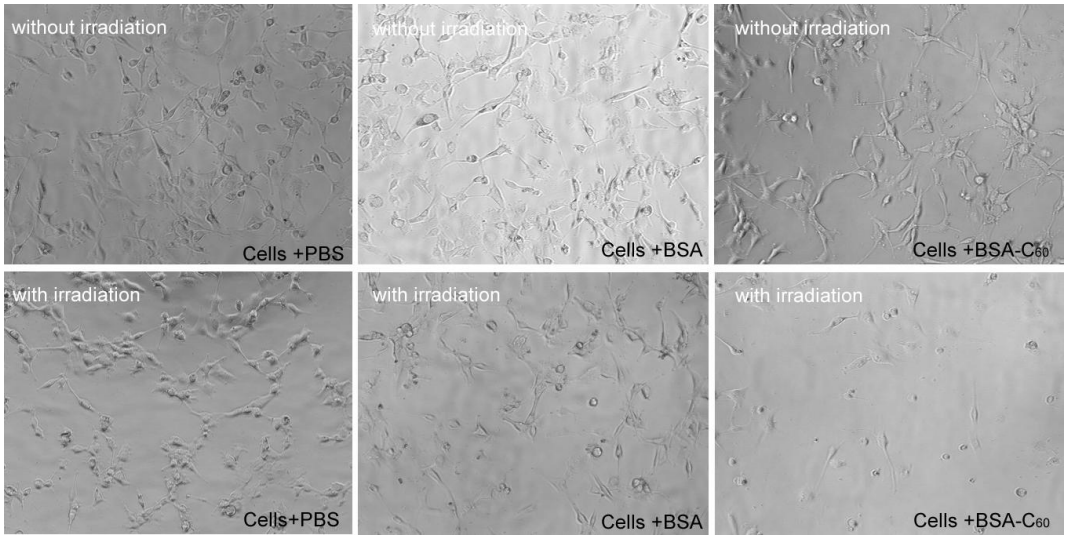

Fig. S6 Phase contrast micrographs of U87 cell line cultured for 48 h in the presence of PBS, 20 μM BSA, and 20 μM BSA containing 24.16 μg mL^{-1} C_{60} without irradiation and with visible light irradiation.

Table S1. Concentration of dispersed C_{60} in water by different methods

Methods		Concentration of C_{60} in water (μg mL^{-1})	Diameter (nm)	Refs
Direct stirring		0.4–1.5	2–142	8
Ultrasonication		133.9	152	1
Solvent exchange	Tetrahydrofuran	4.8	3–78	9
	Toluene	87.3	60–70	10
	Benzene	181.4	1–72	11
Encapsulation	γ–Cyclodextrin	—[a]	115–136	12
	Tween–80	250	33	13
	Phosphatidylcholine	144	12–38	3
	Span 60	100.1	170	14
	Triton X–10	2210	10–100	15
Solubilization (BSA)		241.6	20–60	This work

a: the concentration was not provided in the reference.

Reference

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