

SUPPLEMENTARY INFORMATION

pH Responsive Smart Carrier of [60] Fullerene with 6-Amino-Cyclodextrin Inclusion Complex for Photodynamic Therapy

Kazuyuki Nobusawa*^c, Motofusa Akiyama^d, Atsushi Ikeda^c and Masanobu Naito*^{a,b,c}

^a *TU–NIMS Joint Research Center, School of Materials Science and Engineering, Tianjin University, 92 Weijin Road, Nankai District, Tianjin 300072, P. R. China, and National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-044, Japan.*

E-mail: NAITO.Masanobu@nims.go.jp

Tel: + 81 029 860 4783

^b *JST PRESTO*

^c *Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan.*

E-mail: n-kazuyu@ms.naist.jp

Fax: +81 743 72 6018; Tel: +81 743 72 6017

^d *Department of Applied Chemistry, Faculty of Science and Engineering, Chuo University, 1-13-27 Kasuga, Bunkyo-ku, Tokyo 113-8551, Japan.*

Synthesis details

6-iodo- γ -cyclodextrin

The halogenation of cyclodextrin was carried out using the Vilsmerier–Haack reagent $[(\text{CH}_3)_2\text{NCHI}]$. To stirred solution of Ph_3P (20.20 g, 77.01 mmol) in dry DMF (40 mL) was carefully added I_2 (19.55 g, 77.03 mmol) over 30 min. After the solution was heated to 60 °C, dry γ -cyclodextrin (5.00 g, 3.85 mmol) was then added to this dark brown solution, and the temperature was raised to 70 °C. At this temperature, the mixture was vigorously stirred under Ar atmosphere for 24 h. The reactant solution was then concentrated under reduced pressure. After cooling to 0 °C, NaOMe in MeOH (3 M) was then added to this solution, and the resulting solution was further stirred at r.t. for 30 min. The precipitate was occurred from the solution poured into excess of MeOH, and the precipitate was purified by Soxhlet extractor with MeOH, and dried under high vacuum to yield 6.76 g (81%): ^1H NMR (400 MHz, CD_3SOCD_3) δ 3.39–3.44 (m, overlapped with DMSO), 3.59–3.63 (m, 16H), 3.82 (d, $J = 8$ Hz, 8H), 5.03 (d, $J = 3$ Hz, 8H), 5.98–6.00 (ss, 16H); MALDI-TOFMS m/z 2198.1 $[\text{M} + \text{Na}]^+$.

6-azido- γ -cyclodextrin

To 6-iodo- γ -cyclodextrin (2.21 g, 1.02 mmol) in dry DMF (20 mL) was added NaN_3 (0.73 g, 11 mmol), the resulting suspension was stirred at 60 °C under Ar atmosphere for 20 h. After cooling to r.t., the suspension was poured into water. The resulting white precipitate was filtered carefully, and washed with water. The product was dried under high vacuum to yield 1.42 g (93%): ^1H NMR (400 MHz, CD_3SOCD_3) δ 3.38–3.43 (m, overlapped with DMSO), 3.54–3.61 (m, 16H), 3.71–3.74 (m, 16H), 4.94 (d, $J = 3$ Hz, 8H), 5.89 (d, $J = 2$ Hz, 8H), 5.95 (d, $J = 7$ Hz, 8H); MALDI-TOFMS m/z 1519.0 $[\text{M} + \text{Na}]^+$.

6-amino- γ -cyclodextrin (ACD)

To 6-azido- γ -cyclodextrin (502 mg, 0.335 mmol) in DMF (6 mL) was added Ph_3P (1.58 g, 6.01 mmol). The evolution of N_2 was observed with the formation of phosphoranimine compounds. The solution was vigorously stirred for 2 h until finish of the evolution of N_2 . Concentrated aqueous NH_3 (28%, 1.64 mL) was added dropwise to the solution. After a few minutes, the reaction mixture turned into an off-white suspension, and was further stirred at r.t. for 24 h. The resulting suspension was concentrated under reduced pressure, and then the precipitate was occurred by

addition of EtOH. The precipitate was washed by hot EtOH, and dried under high vacuum to yield 413 mg (96%). Before NMR characterization, the materials were taken up in water and the pH was carefully brought down to 5 with 1 M HCl, and solution was evaporated under high vacuum: ^1H NMR (400 MHz, D_2O) δ 3.20–3.25 (m, 8H, H_6), 3.38–3.42 (m, 8H, H_6), 3.58 (t, $J = 9.6$ Hz, 8H, H_4), 3.67 (dd, $J = 10.0, 3.6$ Hz, 8H, H_2), 3.96 (t, $J = 9.6$ Hz, 8H, H_3), 4.07–4.11 (m, 8H, H_5), 5.20 (d, $J = 3.6$ Hz, 8H, H_1); ^{13}C NMR (400 MHz, D_2O) δ 103.24 (C_1), 83.63 (C_4), 74.59 (C_3), 74.28 (C_2), 71.12 (C_5), 42.97 (C_6); MALDI-TOFMS m/z 1289.8 [$\text{M} + \text{H}$] $^+$; Anal. Calcd. for $\text{C}_{48}\text{H}_{88}\text{N}_8\text{O}_{32}$: C, 43.50; H, 7.00; N, 8.46. Found: C, 43.75; H, 6.75; N, 8.24.

References

1. P. R. Ashton, R. Königer, J. F. Stoddart, D. Alker and V. D. Harding, *J. Org. Chem.*, 1996, **61**, 903–908.
2. H. Yamamura, K. Suzuki, K. Uchibori, A. Miyagawa, M. Kawai, C. Ohmizo and T. Katsu, *Chem. Commun.*, 2012, **48**, 892–894.

Experimental details

Preparation of the ACD/ C_{60} inclusion complex

ACD/ C_{60} inclusion complex were prepared by high-speed vibration milling technique. Mixtures of C_{60} (2.1×10^{-6} mol) and ACD (8.4×10^{-6} mol) were placed in an agate capsule together with two agate mixing balls. They were mixed vigorously by mixing at 1800 rpm for 20 min using a high-speed vibration mill (MM200, Retsch Co. Ltd.). The solid mixtures were dissolved in 2.0 mL of an aqueous solution following centrifugation ($17970 \times g$, 20 min), all nondispersed C_{60} were removed from the solutions.

Photodynamic activity of the ACD/ C_{60} complex

The cells as human cervical cancer HeLa cells, were seeded on 48-well plates as culture plates at a density of 3.4×10^4 cells cm^{-2} in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 5% fetal calf serum at 37 °C in 5% CO_2 . After growing overnight and changing from DMEM to 200 μL of the C_{60} complex solution prepared using PBS buffer. The sample for the experiment in pH 6.4 was prepared through adding 10 μL of the complex solution to 190 μL of PBS buffer just before the incubation because of avoiding the aggregation of C_{60} . After 30-min incubation at 37 °C, changing from complex solution to DMEM, the cultures were exposed to light

(400–500 nm) at 25 °C for 30 min. The power of light at the cellular level was 54 mW cm⁻². Following irradiation, the plates were incubated for 24 h at 37 °C, 5% CO₂. Cell survival was determined using a WST-8 assay (Cell Counting Kit-8: Dojindo Laboratories Co., Kumamoto, Japan).

Transmission electron microscopy (TEM) observation

The intracellular uptake of ACD/C₆₀ in HeLa cells was performed by TEM (HITACHI H-7100, at 75 kV accelerating voltage). HeLa cells were incubated with ACD/C₆₀ (concentration of C₆₀ was 10 μM) for 30 min at 37 °C and pH 6.4, and rinsed twice with PBS. They were digested with trypsinase-EDTA and washed with PBS. Then, the cells were fixed with glutaraldehyde (2.5%, v/v) for 2 h and a further 1 h in a solution of OsO₄ (2%, w/v) at 4 °C. The fixed cells were dehydrated through an ethanol series (30, 50, 70, 90, and 100%) and were incubated for 15 min in each solution. Samples were embedded with Spurr resin and incubated at 60 °C for 48 h. The curing resin containing cells was cut into 100 nm thick slices, which were deposited on a copper grid. In some cases the samples were stained with uranium and lead salts prior to observing by TEM.

Supplementary data

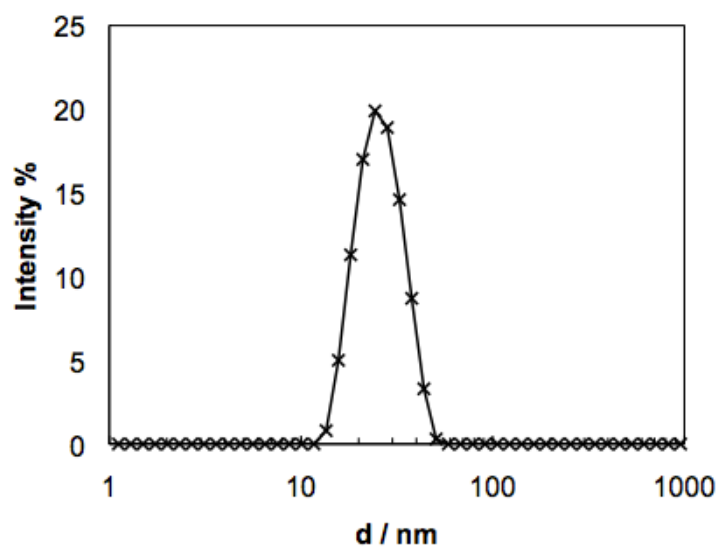


Fig. S1 Size distribution from DLS measurement of C₆₀ aggregate incorporated with ACD in aqueous solution at pH 5.0.

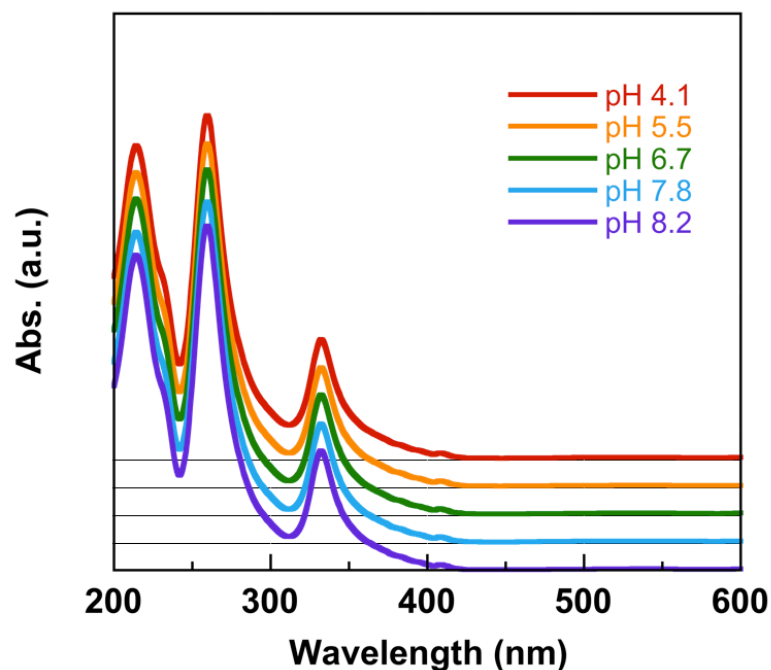


Fig. S2 Changes in UV-vis absorption spectra of γ -CD/ C_{60} in water with varying pH.

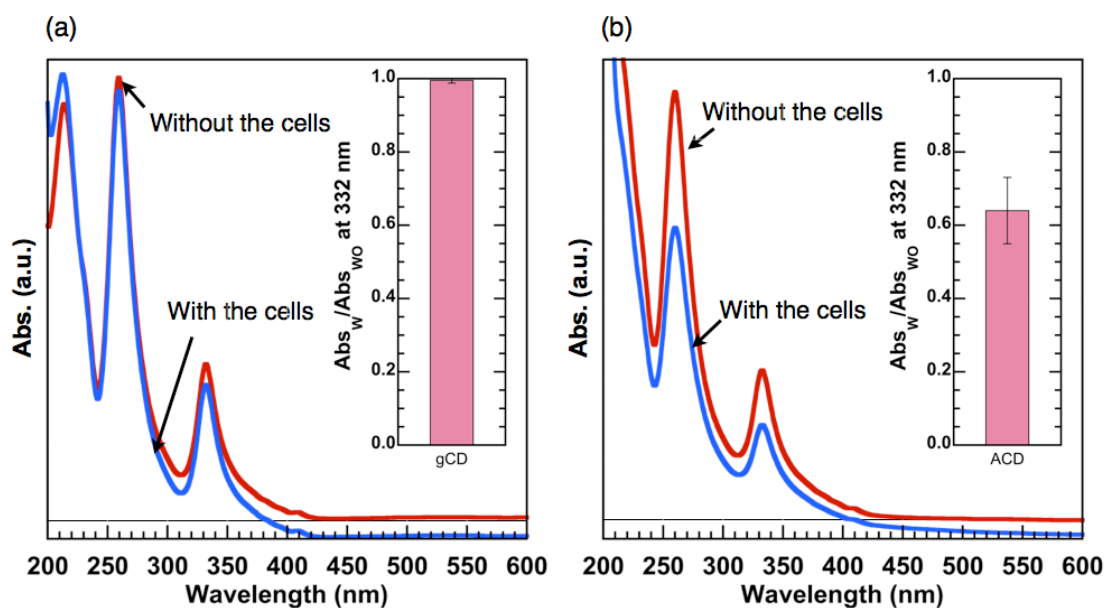


Fig. S3 UV-vis absorption spectra of (a) γ -CD/ C_{60} and (b) ACD/ C_{60} after incubation for 30 min with and without HeLa cells in pH 7.4 PBS buffer at 37 °C. Inset figure showed the absorbance ratio of with/without HeLa cells at 332 nm. [C_{60}] = 10 μ M.