Supplementary Information for:

Cellular uptake of octaarginine-conjugated tetraarylporphyrin included by per-O-methylated β-cyclodextrin

Hiroaki Kitagishi, Satoshi Hatada, Toshiaki Itakura, Yuki Maki, Yasuaki Maeda and Koji Kano

Department of Molecular Chemistry and Biochemistry, Faculty of Science and Engineering, Doshisha University, Tatara, Kyotanabe, Kyoto 610-0321, Japan.
Fig. S1  HPLC charts of (A) R8-TPP (1) and (B) R8-TPP (2) recorded at 230 nm (red) and 410 nm (blue). A Wakosil-II 5C18-AR (4.6 mm x 250 mm) reverse phase column was used for the analyses. Flow rate of the eluent was 0.5 mL / min. The samples were eluted with a gradient solvent system using CH$_3$CN/0.1% TFA in water started at 30% (v/v) and linearly increased at the rate of 1%/min. The green lines represent ratio of CH$_3$CN in the eluent. From the chromatograms monitored at 230 nm, the purities of these compounds are estimated to be above 98%. 

Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
This journal is © The Royal Society of Chemistry 2013
Fig. S2  UV-Vis spectra of 1 (2 × 10⁻⁶ M) before (blue) and after the addition of 5% FBS (red). The spectra were recorded (A) in the absence and (B) presence of TMe-β-CD (6 × 10⁻⁶ M) in phosphate buffer saline (PBS) at pH 7.4 and 25 °C. The spectral change indicates that the serum protein, mainly serum albumin, in FBS binds R8-TPP to form the R8-TPP/protein complex that shows $\lambda_{\text{max}} = 420$ nm. The R8-TPP/protein complex formed even in the presence of TMe-β-CD.
**Fig. S3** Fluorescence micrographs of the HeLa cells after incubation with 1 (2 × 10⁻⁶ M) and TMe-β-CD (6 × 10⁻⁶ M) at 4 °C for 30 min, then with DAPI at 37 °C for 30 min. Luminescence was shown as red (excited at 435 ± 25 nm) and blue (excited at 360 ± 40 nm).

**Fig. S4** Fluorescent micrographs of HeLa cells after incubation with (A) 2 (2 × 10⁻⁶ M) in DMEM-FBS, (B) 2 (2 × 10⁻⁶ M) and TMe-β-CD (6 × 10⁻⁶ M) in DMEM-FBS, (C) 2 (2 × 10⁻⁶ M) in OPTI-MEM, and (D) 2 (2 × 10⁻⁶ M) and TMe-β-CD (6 × 10⁻⁶ M) in OPTI-MEM at 37 °C for 2 h, then with DAPI at 37 °C for 30 min. Luminescence was shown as red (excited at 435 ± 25 nm) and blue (excited at 360 ± 40 nm).
Cell viability determined by MTT assay after the incubation with TMe-β-CD (5 x 10^{-6}, 5 x 10^{-5} and 1 x 10^{-3} M) in DMEM-FBS or OPTI-MEM under the dark condition. Each bar represents the mean ± SD of the data obtained from at least three experiments. The data indicates that free TMe-β-CD does not show any cytotoxic effect under these incubation conditions.
Fig. S6  Dark and photo cytotoxicity of R8-TPP without TMe-β-CD. The cells were incubated in DMEM-FBS or OPTI-MEM containing R8-TPP (1) (5 × 10⁻⁶ M) at 37 °C for 2 h, washed with PBS, and then soaked in fresh DMEM-FBS. The cells were then treated with filtered light at 310-510 nm for 15 min for measuring photo-induced cytotoxicity. Cell viability was measured by MTT assay. Each bar represents the mean ± SD of the data obtained from at least three experiments. The asterisks indicate statistical difference from other data as determined by Student’s t-test, **P < 0.01. The data indicates that (1) R8-TPP shows slight cytotoxicity even in the absence of light irradiation and (2) endosome trapped R8-TPP that was found in the serum-containing medium (DMEM-FBS) (Fig 5A-D) and self-aggregated R8-TPP on the cell surface in the absence of both TMe-β-CD and serum (Fig 5E and F) do not show significant cytotoxic effect as compared with the cytosol dispersed one (Fig 5G and H).