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Electronic Supplementary Information (ESI)

Formulation of water-dispersible hydrophobic compound nanocomplexes using polypeptides via supramolecular approach using a high-speed vibration milling technique

Shogo Kawamura,[a,#] Riku Kawasaki,[a,#,*] Shodai Hino,[a,b,#] Keita Yamana,[a] Masafumi Okuno,[a] Takuro Eto[a] and Atsushi Ikeda[a,*]

a) Program of Applied Chemisty, Graduate School of Advanced Science and Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashi Hiroshima, 739-8527, Japan.

b) Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology, AIST; 1-8-31 Midorigaoka, Ikeda, 563-8577, Japan.

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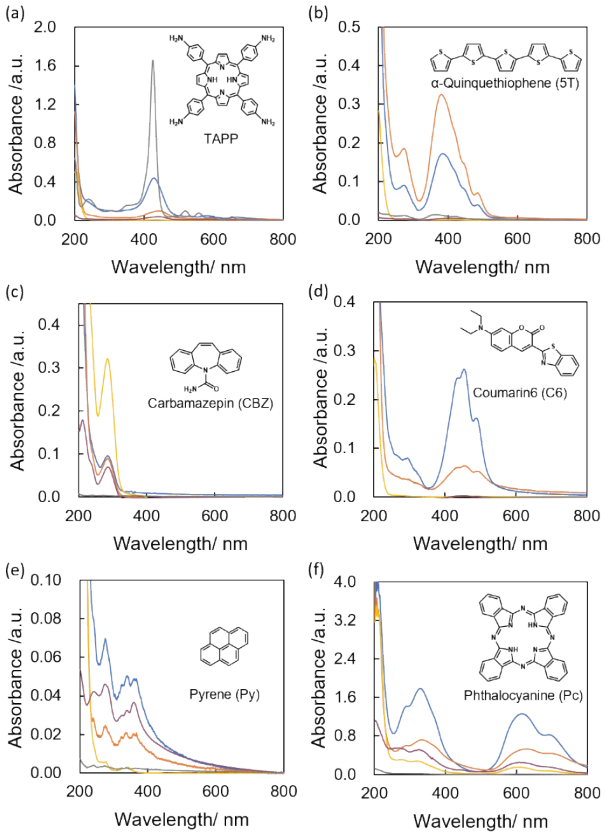

Fig. S1 UV-Vis absorption spectra of prepared complexes. (a) PLL/TAPP (blue), PGA/TAPP (orange), Col/TAPP (yellow), Pul/TAPP (Purple), and TMe-β-CDx/TAPP (gray). (b) PLL/5T (blue), PGA/5T (orange), Col/5T (yellow), Pul/5T (Purple), and TMe-β-CDx/5T (gray). (c) PLL/CBZ (blue), PGA/CBZ (orange), Col/CBZ (yellow), Pul/CBZ (Purple), and TMe-β-CDx/CBZ (gray). (d) PLL/C6 (blue), PGA/C6 (orange), Col/C6 (yellow), Pul/C6 (Purple), and TMe-β-CDx/C6 (gray). (e) PLL/Py (blue), PGA/Py (orange), Col/Py (yellow), Pul/Py (Purple), and TMe-β-CDx/Py (gray). (f) PLL/Pc (blue), PGA/Pc (orange), Col/Pc (yellow), Pul/Pc (Purple), and TMe-β-CDx/Pc (gray). All absorption spectra of these complexes were measured in H_2O at 25 °C using a 1-mm cell pathlength.

	Conc. (μM)	D_{hy} (nm)	PDI
PLL/TAPP	300	210 ± 4	0.27
PGA/TAPP	50	570 ± 20	0.41
Col/TAPP	2	110 ± 10	0.82
Pul/TAPP	30	330 ± 10	0.29
TMe-β-CDx/TAPP	280	—	—
PLL/5T	760	530 ± 10	0.22
PGA/5T	1400	380 ± 5	0.23
Col/5T	0	—	—
Pul/5T	20	610 ± 50	0.34
TMe-β-CDx/5T	63	—	—
PLL/CBZ	520	670 ± 20	0.18
PGA/CBZ	490	680 ± 20	0.24
Col/CBZ	1700	180 ± 20	0.26
Pul/CBz	380	990 ± 150	0.62
TMe-β-CDx/CBz	20	—	—
PLL/C6	870	360 ± 6	0.07
PGA/C6	310	740 ± 7	0.21
Col/C6	10	470 ± 40	0.31
Pul/C6	10	370 ± 50	0.36
TMe-β-CDx/C6	0	—	—
PLL/Py	410	340±3	0.01
PGA/Py	250	720±60	0.16
Col/Py	16	410±30	0.38
Pul/C6	370	380 ± 10	0.37
TMe-β-CDx/C6	120	—	—
PLL/Pc	—	—	—
PGA/Pc	—	—	—
Col/Pc	—	—	—
Pul/Pc	—	—	—
TMe-β-CDx/Pc	—	—	—

Table S1 Basic characterization of complexes with polypeptide.

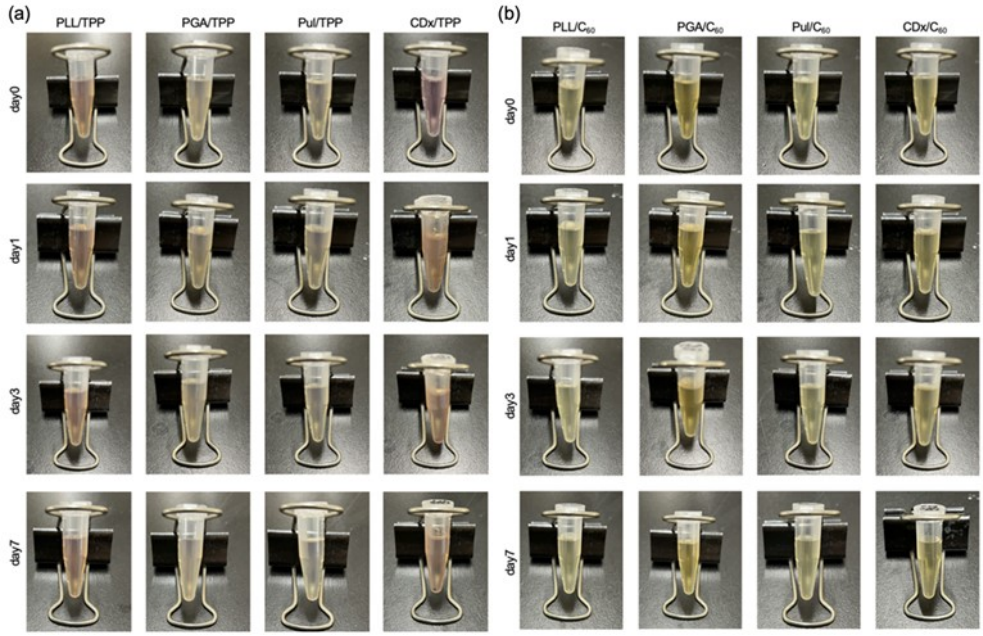

Fig. S2 Representative photographic images of dispersion. (a) The complex of TPP with PLL, PGA, Pul and CDx were incubated at 37 °C for 7 days and the photos was taken at each time point (0, 1, 3, and 7 days). (b) The complex of C_{60} with PLL, PGA, Pul and CDx were incubated at 37 °C for 7 days and the photos was taken at each time point (0, 1, 3, and 7 days).

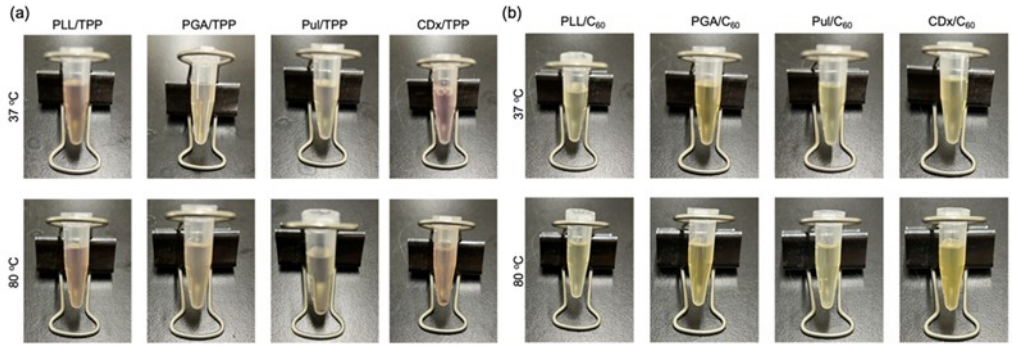

Fig. S3 Representative photographic images of dispersion with varying incubation temperature (37 °C and 80 °C). (a) The complex of TPP with PLL, PGA, Pul and CDx were incubated at 37 °C (upper panel) or 80 °C (bottom panel) for 2 h and the photos was taken. (b) The complex of C_{60} with PLL, PGA, Pul and CDx were incubated at 37 °C (upper panel) or 80 °C (bottom panel) for 2 h and the photos was taken.

Fig. S4 Stability of prepared water-dispersible nanocomplexes. (a) Long-term stability of TPE complexes with biopolymers (PLL, blue; PGA, orange; Col, yellow; and Pul, purple). (b) Representative photographic images of dispersion. The complex of TPE with PLL, PGA, Col, and Pul were incubated at 25 °C for 7 days and the photos was taken at each time point (0, 1, 3, and 7 days). Representative photographic images of dispersion with varying incubation temperature (37 °C and 80 °C). (c) Stability of TPE complexes with biopolymers against thermal stimuli at 80 °C (PLL, blue; PGA, orange; Col, yellow; and Pul, purple). (d) Representative photographic images of dispersion

with varying incubation temperature. The complex of TPE with PLL, PGA, Col, and Pul were incubated at 37 ºC (upper panel) or 80 ºC (bottom panel) for 2 h and the photos was taken.

Table S2 Basic characterization of polypeptide/THPP complexes.

	Conc. (μM)	D_{hy} (nm)	PDI	ζ-potential (mV)
PLL/THPP	3200	300 ± 4	0.09	+59 ± 1
PGA/THPP	800	220 ± 3	0.14	-56 ± 1

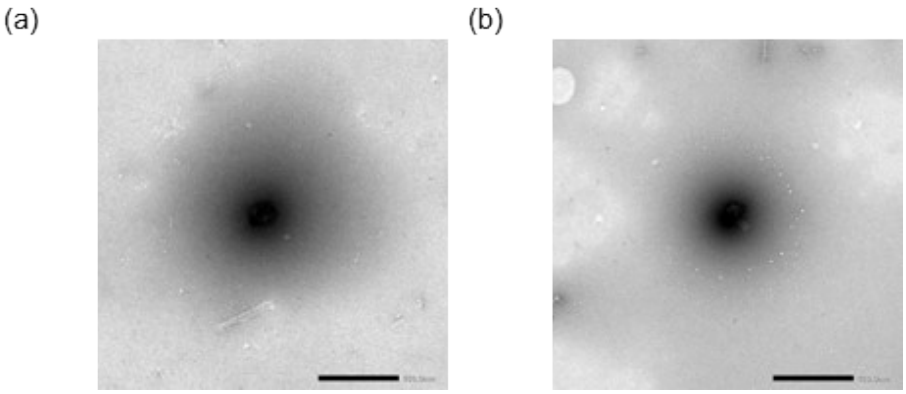

Fig. S5 Representative morphology of a THPP complex with polypeptide after ultrasonic treatment observed under a transmission electron microscope (a, PLL/THPP; b, PGA/THPP). The samples were stained with 3% ammonium molybdate. Scalebar measures 500 nm.

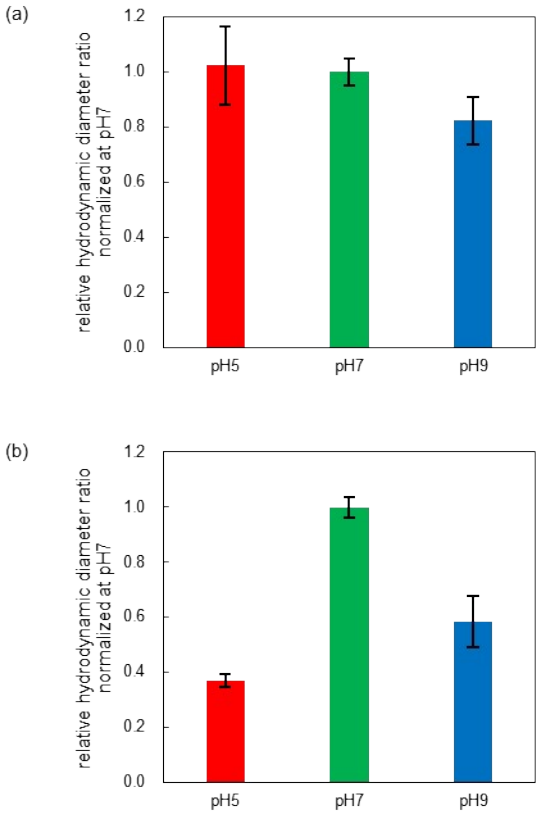

Fig. S6 Change in hydrodynamic diameter with pH change. PLL/THPP (a) and PGA/THPP complexes (b) were incubated in water at different pH (pH 5, red; pH 7, green; pH 9, blue) prepared with hydrochloric acid or sodium hydroxide solutions ([THPP]=15 μM). Error bars represent the standard deviations.

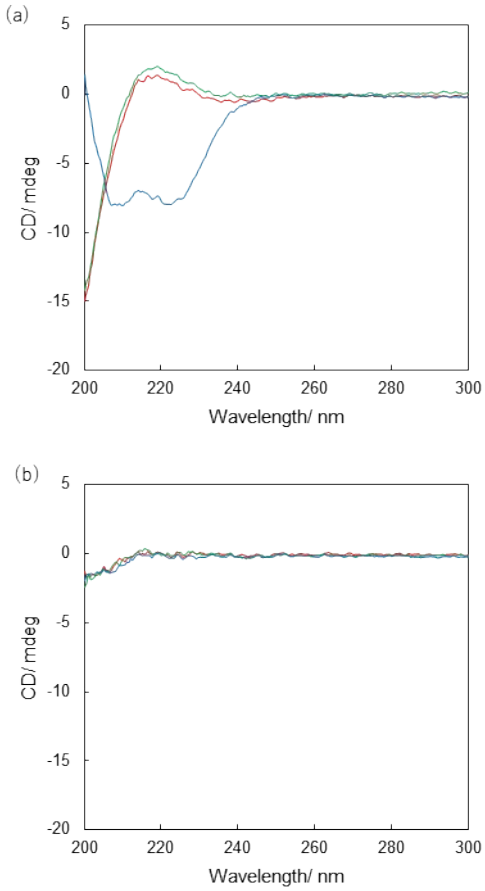

Fig. S7 Change in CD spectrum as a function of pH. PLL/THPP (a) and PGA/THPP complexes (b) were incubated in water at different pH (pH 5, red; pH 7, green; pH 9, blue) prepared with hydrochloric acid or sodium hydroxide solutions ([THPP]=15 μM).

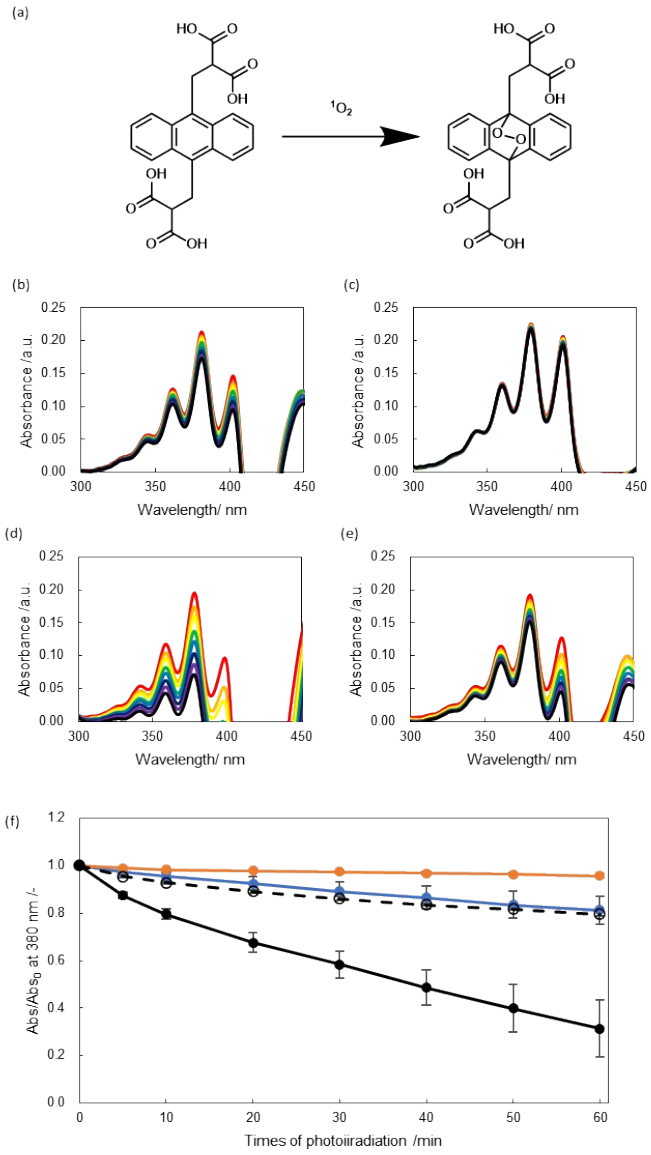

Fig. S8 (a) Conversion of 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) to a corresponding endoperoxide via oxidation by a singlet oxygen ($^1O_2$). (b)-(e) Time-dependent bleaching of ABDA caused by the generation of a singlet oxygen from (b) PLL/THPP complexes, (c) PGA/THPP complexes, (d) DMSO/THPP solution, (e) DMSO/THPP solution with PLL, and upon photoirradiation ($\lambda > 620$ nm, 15 mW cm^{-2}) for 0 (red line), 5 (orange line), 10 (yellow line), 20 (green line), 30 (blue line), 40 (dark blue line), 50 (purple line), and 60 (black line) min. [THPP] = 15 μM, [ABDA] = 25 μM. Experiments were conducted under an oxygen atmosphere at 25°C. $n = 3$. (f) Time-dependent changes in ABDA absorption at 380 nm (Abs_0: initial absorbance). PLL/THPP complexes (blue line), PGA/THPP complexes (orange line), DMSO/THPP solution (black line), and DMSO/THPP solution with PLL (black dashed line). All data represent the mean values of three independent experiments. Error bars represent the standard deviations.

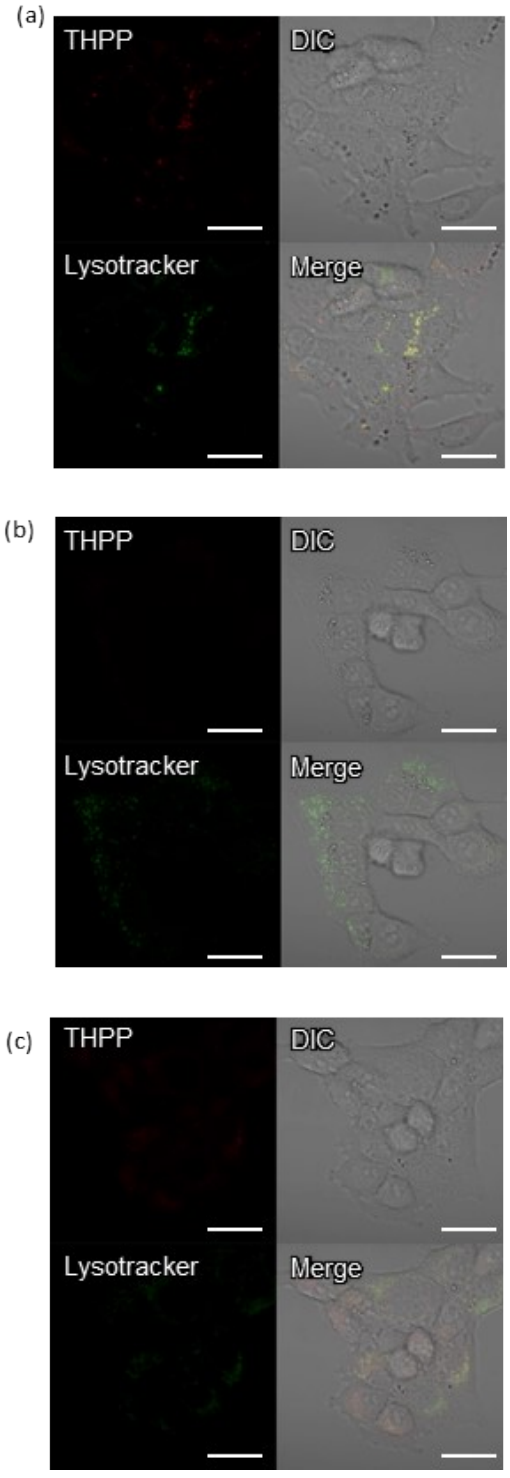

Fig. S9 Subcellular distribution of delivered THPP. Colon-26 cells were co-incubated with (a) PLL/THPP complex, (b) PGA/THPP complex, and (c) DMSO/THPP solution ([THPP] = 1.0 μM) for 4 h. Lysosomes were visualized using LysoTracker Green DND-26. The cells were observed using a confocal laser scanning microscope. Scalebars represent 30 μm.