Electronic Supplementary Information (ESI)

Photo-Responsive Azobenzene Interactions Promote Hierarchical Self-Assembly of Collagen Triple-Helical Peptides to Various Higher-Order Structures

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Experimental Procedures and Analyses

Syntheses of C_mAzo-OH (*m*=6,12).

C_mAzo-OH (*m*=6, 12) were synthesized as follows. 4-(4-hexyloxyphenylazo)benzoic acid hexyl ester was firstly prepared by reaction of 4-(4-hydroxyphenylazo)benzoic acid (1.25 mmol) and 1-bromohexane(10.0 mmol) in DMF with K_2CO_3 (1.25 mmol) and 18-crown-6-ether (0.125 mmol) at room temperature for 24 h, and then the reaction mixture was pored into water and extracted with *n*-hexane and finally recrystallized with *n*-hexane. 4-(4-dodecyloxyphenylazo)benzoic acid dodecyl ester was prepared using 1-bromododecane in place of 1-bromohexane by the same manner and stoichiometry. These esters thus obtained were allowed to hydrolysis with ethanolic NaOH in THF giving the corresponding objectives. The chemical structure was determined by ^{1}H NMR spectroscopy.

C_6Azo-OH (4-(4-hexyloxyphenylazo)benzoic acid) : ^{1}H NMR (DMSO-d_6, TMS) 0.8-1.0 ppm (3H, -CH_3), 1.3-1.9 ppm (8H,-$CH_2(CH_2)_4CH_3$), 4.0-4.1 ppm (2H, -CH_2CH_2O-), 7.0-8.2 ppm (8H, Ar : aromatic ring of azobenzene), 13.1-13.2 ppm (1H, Ar-COOH).

C_{12}Azo-OH (4-(4-dodecyloxyphenylazo)benzoic acid): ^{1}H NMR (DMSO-d_6, TMS) 0.8-1.0 ppm (3H, -CH_3), 1.3-1.9 ppm (20H, -$CH_2(CH_2)_{10}CH_3$), 4.0-4.1 ppm (2H, -CH_2CH_2O-), 7.0-8.2 ppm (8H, Ar : aromatic ring of azobenzene), 13.1-13.2 ppm (1H, Ar-COOH).

Azo-$(GPO)_n$, Azo-*deg*-$(GPO)_5$, C_{12}Azo-$(GPO)_5$ (*m*=3-10) (see Figures S1 and S2)

Azo-$(GPO)_3$: MALDI-TOFMS 1029.6 $[M+H]^+$/1028.1 $[M+H]^+_{Theory}$.
^{1}H NMR (D2O, DSS) 1.7-2.6 ppm (18H : Pro-β, Pro-γ, Hyp-β), 3.3-4.3 ppm (18H : Pro-δ, Hyp-δ, Gly-α), 4.5-4.8 ppm (9H : Hyp-γ, Hyp-α, Pro-α), 6.6 ~ 8.0 ppm (9H : aromatic ring of azobenzene).

Azo-$(GPO)_4$: MALDI-TOFMS 1296.9 $[M+H]^+$ /1295.4 $[M+H]^+_{Theory}$.
^{1}H NMR (D2O, DSS) 1.7-2.6 ppm (24H : Pro-β, Pro-γ, Hyp-β), 3.3-4.3 ppm (24H : Pro-δ, Hyp-δ, Gly-α), 4.5-4.8 ppm (12H : Hyp-γ, Hyp-α, Pro-α), 6.6-8.0 ppm (9H : aromatic ring of azobenzene).

Azo-(GPO)$_5$: MALDI-TOFMS 3.0 [M+H]+ / 1562.7 [M+H]+Theory.
1H NMR (D_2O, DSS) 1.7-2.6 ppm (30H : Pro-β, Pro-γ, Hyp-β), 3.3-4.3 ppm (30H : Pro-δ, Hyp-δ, Gly-α), 6.6-8.0 ppm (9H : aromatic ring of azobenzene).

Azo-(GPO)$_6$: MALDI-TOFMS 1831.0 $[M+H]^+$/1829.9 $[M+H]^+_{Theory}$.
^{1}H NMR (D_2O, DSS) 1.7-2.6 ppm (36H : Pro-β, Pro-γ, Hyp-β), 3.3-4.3 ppm (36H : Pro-δ, Hyp-δ, Gly-α), 6.6-8.0 ppm (9H : aromatic ring of azobenzene).

Azo-(GPO)$_7$: MALDI-TOFMS 2098.9 $[M+H]^+$/2097.2 $[M+H]^+_{Theory}$.
^{1}H NMR (D_2O, DSS) 1.7-2.6 ppm (42H : Pro-β, Pro-γ, Hyp-β), 3.3-4.3 ppm (42H :Pro-δ, Hyp-δ, Gly-α), 6.6-8.0 ppm (9H : aromatic ring of azobenzene).

Azo-(GPO)$_8$: MALDI-TOFMS 2365.4 $[M+H]^+$/2364.5 $[M+H]^+_{Theory}$.
^{1}H NMR (D_2O, DSS) 1.7-2.6 ppm (48H : Pro-β, Pro-γ, Hyp-β), 3.3-4.3 ppm (48H : Pro-δ, Hyp-δ, Gly-α), 6.6-8.0 ppm (9H : aromatic ring of azobenzene).

Azo-(GPO)$_9$: MALDI-TOFMS 2632.5 $[M+H]^+$/2631.8 $[M+H]^+_{Theory}$.
^{1}H NMR (D_2O, DSS) 1.7-2.6 ppm (54H : Pro-β, Pro-γ, Hyp-β), 3.3-4.3 ppm (54H : Pro-δ, Hyp-δ, Gly-α), 6.6-8.0 ppm (9H : aromatic ring of azobenzene).

Azo-(GPO)$_{10}$: MALDI-TOFMS 2900.1 $[M+H]^+$/2899.1 $[M+H]^+_{Theory}$.
^{1}H NMR (D_2O, DSS) 1.7-2.6 ppm (60H : Pro-β, Pro-γ, Hyp-β), 3.3-4.3 ppm (60H : Pro-δ, Hyp-δ, Gly-α), 6.6-8.0 ppm (9H : aromatic ring of azobenzene).

Azo-*deg*-(GPO)$_5$: MALDI-TOFMS 1708.7 $[M+H]^+$/1707.8 $[M+H]^+_{Theory}$.
^{1}H NMR (D_2O, DSS) 1.7-2.7 ppm (30H : Pro-β, Pro-γ, Hyp-β), 3.3-4.3 ppm (40H : deg, Pro-δ, Hyp-δ, Gly-α), 6.6-8.0 ppm (9H : aromatic ring of azobenzene).

C_6Azo-$(GPO)_5$: MALDI-TOFMS 1662.9 $[M+H]^+$/1662.8 $[M+H]^+_{Theory}$.

^1H NMR (D_2O/TFE, DSS) 0.8-1.0 ppm (3H, CH_3- : alkyl chain), 1.2-1.6 ppm (8H, $CH_3(CH_2)_4CH_2$- : alkyl chain), 1.7-2.6 ppm (30H : Pro-β, Pro-γ, Hyp-β), 3.4-4.3 ppm (30H :Pro-δ, Hyp-δ, Gly-α), 7.0-8.0 ppm (8H : aromatic ring of azobenzene).

C_{12}Azo-$(GPO)_5$: MALDI-TOFMS 1747.6 $[M+H]^+$/1746.9 $[M+H]^+_{Theory}$.

^1H NMR (D_2O/TFE, DSS) 0.8-1.0 ppm (3H, CH_3- : alkyl chain), 1.2-1.6 ppm (20H, $CH_3(CH_2)_{10}CH_2$- : alkyl chain), 1.7-2.6 ppm (30H : Pro-β, Pro-γ, Hyp-β), 3.4-4.3 ppm (30H : Pro-δ, Hyp-δ, Gly-α), 7.0-8.0 ppm (8H : aromatic ring of azobenzene).

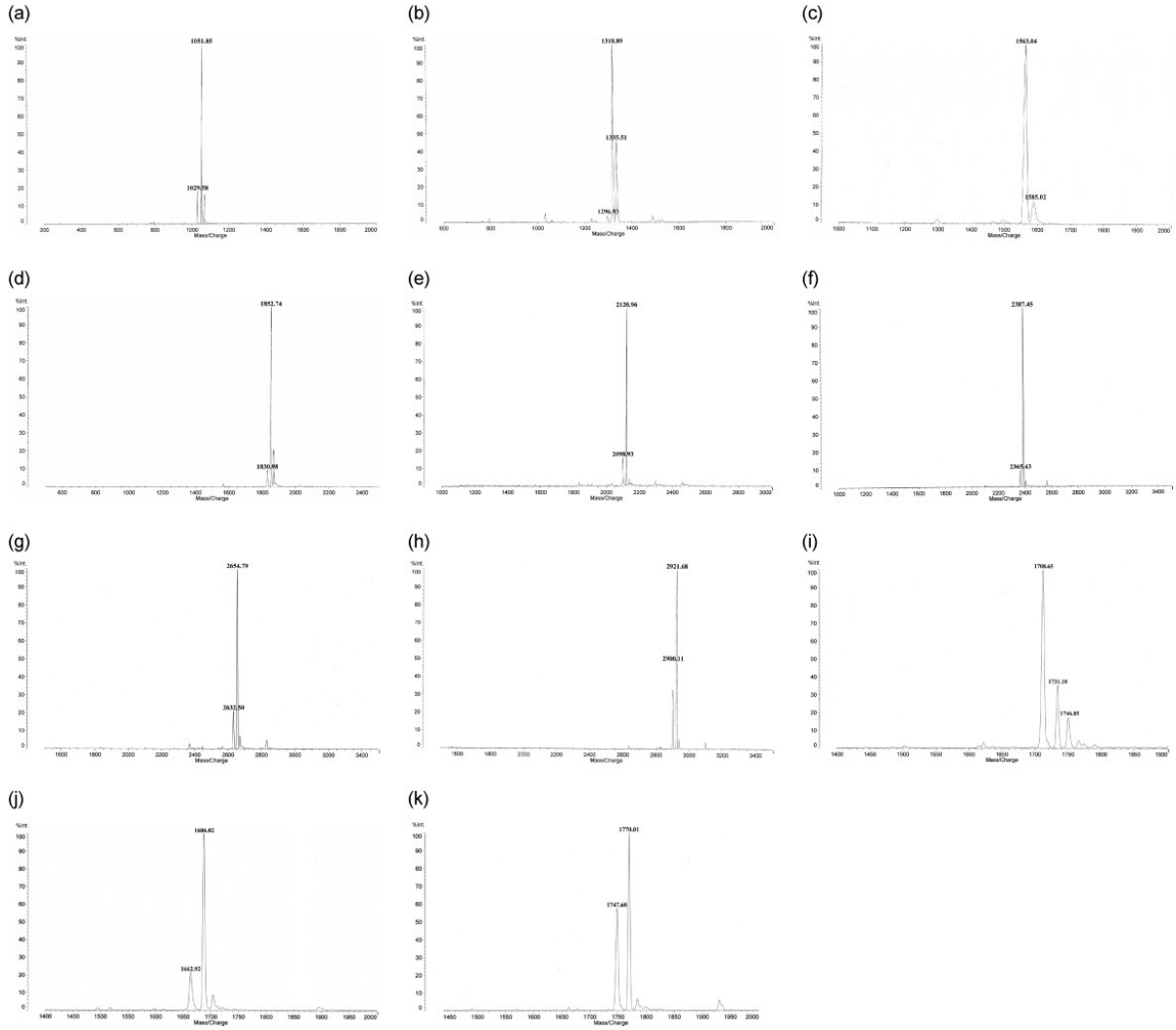

Figure S1. MALDI-TOF MS spectra of **Azo-(GPO)$_n$** (n=3 (a), 4 (b), 5 (c), 6 (d), 7 (e), 8 (f), 9 (g), and 10 (h)), **Azo-*deg*-(GPO)$_5$** (i) and **C$_m$Azo-(GPO)$_5$** (m=6 (j) and 12 (k)).

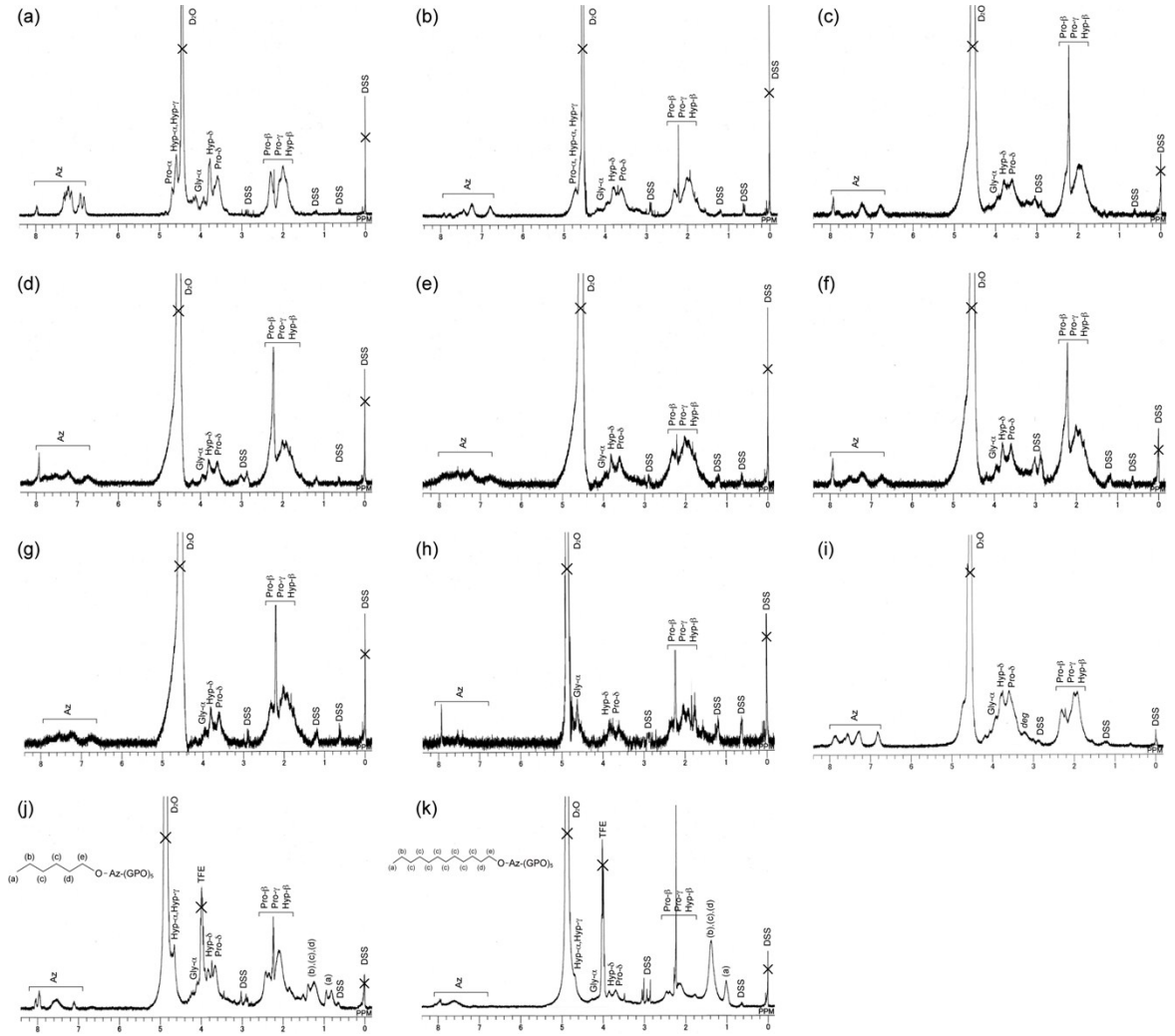

Figure S2. ^{1}H NMR spectra of **Azo-(GPO)$_n$** (*n*=3 (a), 4 (b), 5 (c), 6 (d), 7 (e), 8 (f), 9 (g), and 10 (h)), **Azo-*deg*-(GPO)$_5$** (i) and **C$_m$Azo-(GPO)$_5$** (*m*=6 (j) and 12 (k)).

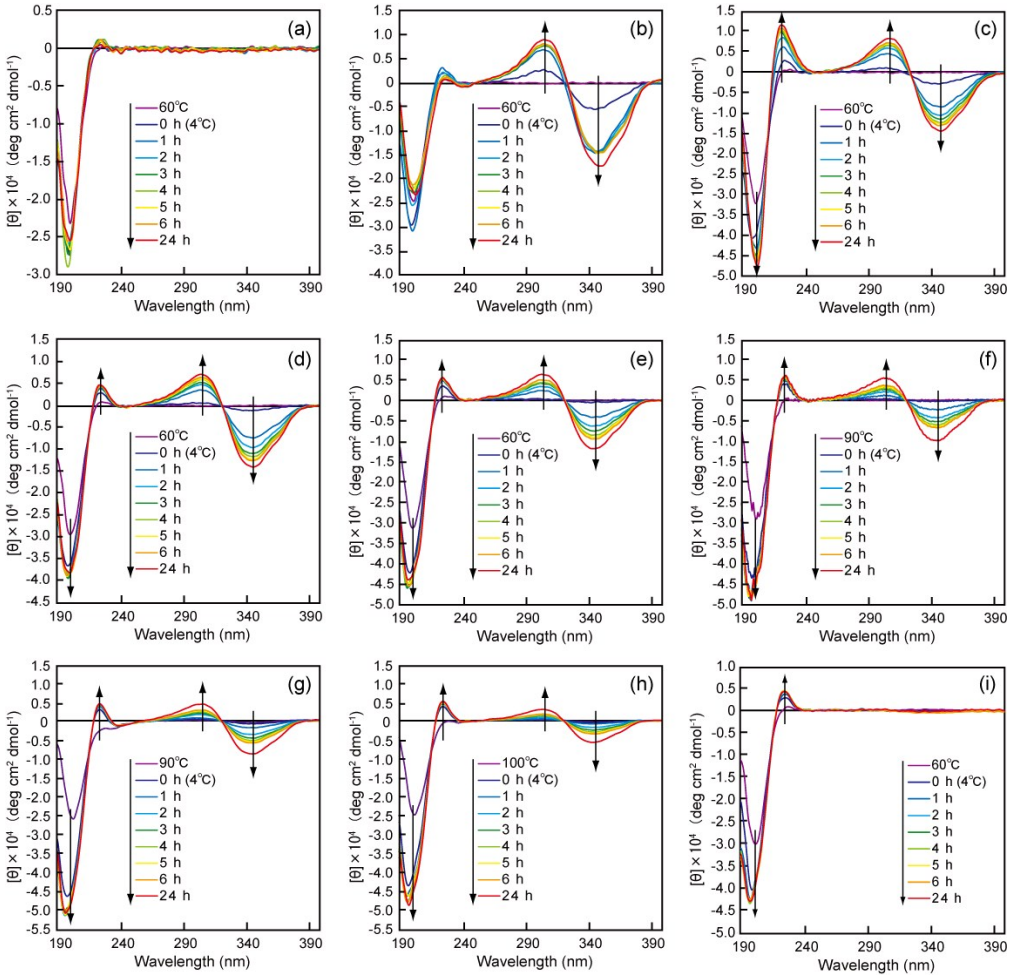

Figure S3. Changes in the CD spectra of **Azo-(GPO)$_n$** (*trans*-form) (*n*=3 (a), 4 (b), 5 (c), 6 (d), 7 (e), 8 (f), 9 (g), and 10 (h)), and **Azo-*deg*-(GPO)$_5$** (*trans*-form) (i) in water upon incubation at 4 °C after the thermal treatment at 60–90 °C, above which the peptides denature.

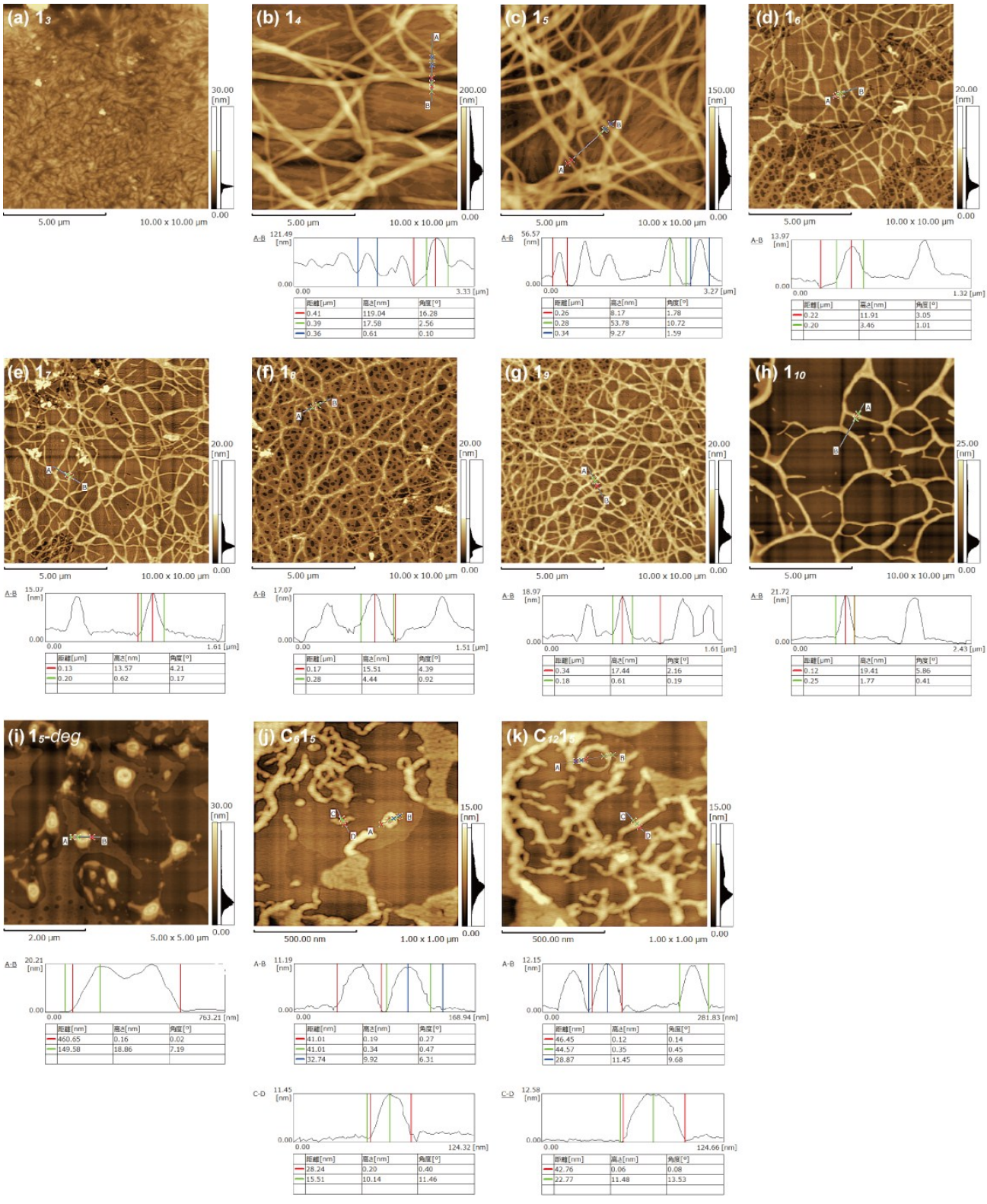

Figure S4. AFM images of **Azo-(GPO)$_n$** (*trans*-form) (n=3 (a), 4 (b), 5 (c), 6 (d), 7 (e), 8 (f), 9 (g), and 10 (h)), **Azo-*deg*-(GPO)$_5$** (*trans*-form) (i), and **C$_m$ Azo-(GPO)$_5$** (*trans*-form) (m = 6 (j) and 12 (k)). The pictures were taken after 24 h incubation at 4 °C.

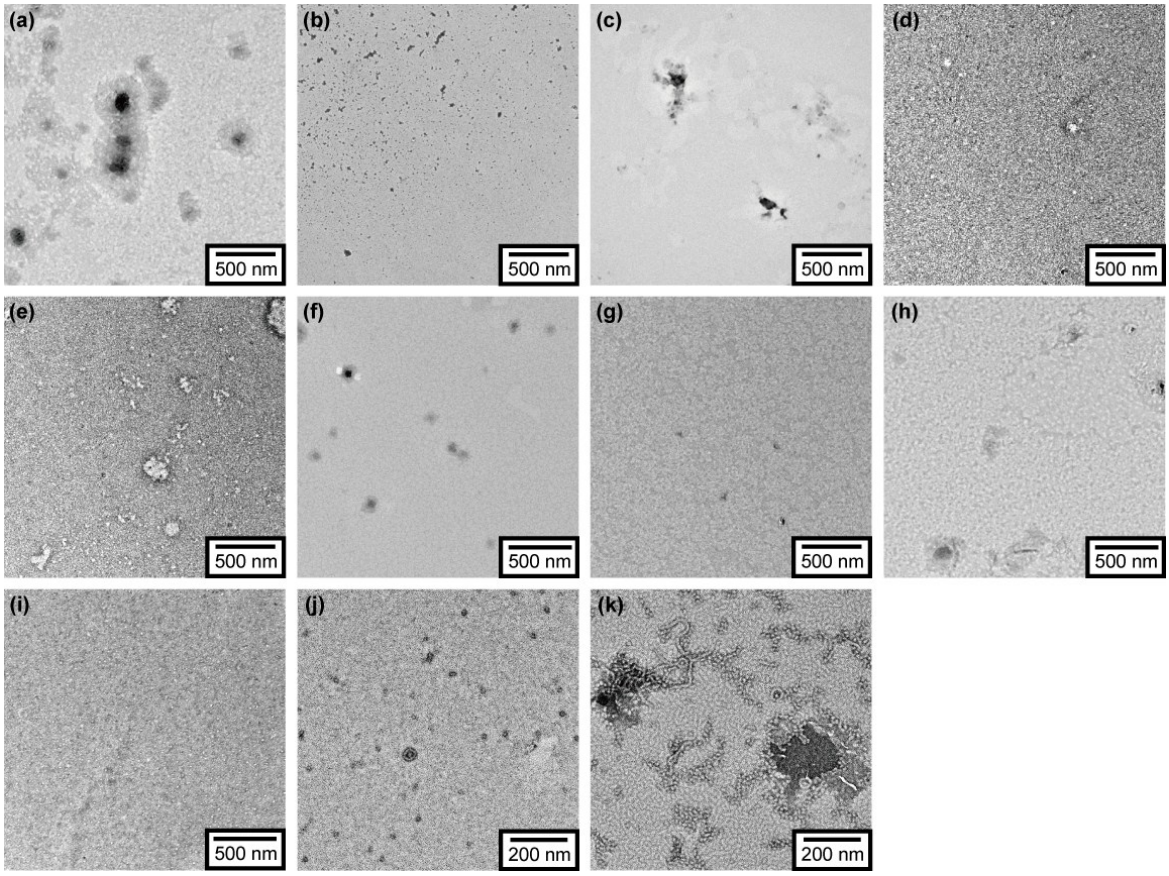

Figure S5. TEM images of **Azo-(GPO)$_n$** (*cis*-form) (*n*=3 (a), 4 (b), 5 (c), 6 (d), 7 (e), 8 (f), 9 (g), and 10 (h)), **Azo-*deg*-(GPO)$_5$** (*cis*-form) (i), and **C$_m$ Azo-(GPO)$_5$** (*trans*-form) (*m* = 6 (j) and 12 (k)). The pictures were taken after 2 h UV irradiation at 4 °C and then stained by phosphotungstic acid.

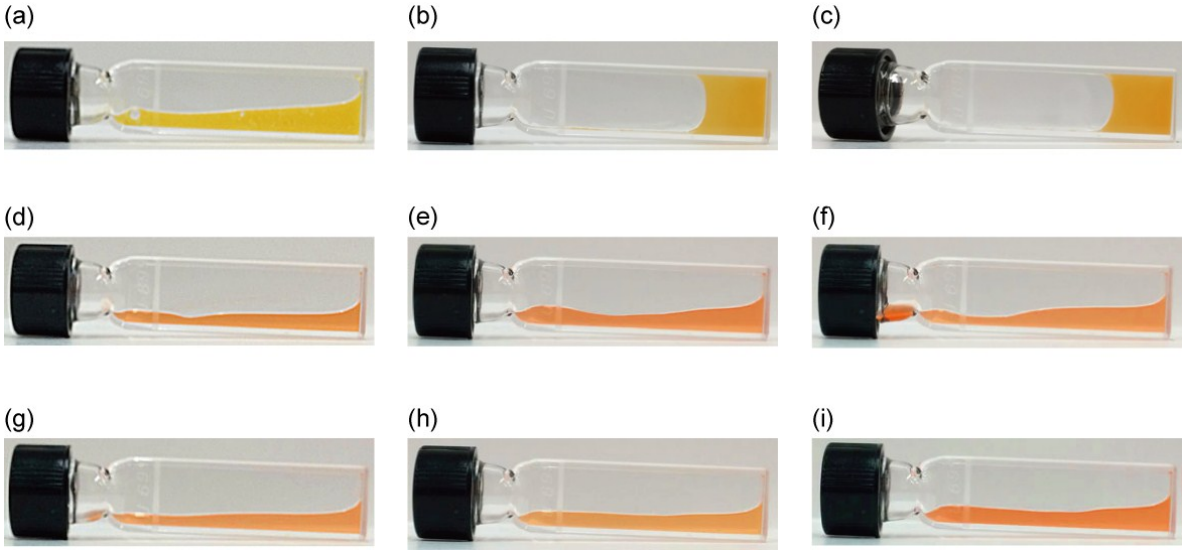

Figure S6. Photographs of **Azo-(GPO)$_n$** (*trans*-form) (*n*=3 (a), 4 (b), 5 (c), 6 (d), 7 (e), 8 (f), 9 (g), and 10 (h)), **Azo-*deg*-(GPO)$_5$** (*trans*-form) (i) aqueous solutions (6 wt%) incubated at 4 °C for 24 h.