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 Cₘₐzo-OH (m=6,12).

Cₘₐzo-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 K₂CO₃ (1.25 mmol) and 18-crown-6-ether (0.125 mmol) at room temperature for 24 h, and then the reaction mixture was poured 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 ¹H NMR spectroscopy.

C₆ₐzo-OH (4-(4-hexyloxyphenylazo)benzoic acid) : ¹H NMR (DMSO-d₆, TMS) 0.8-1.0 ppm (3H, -CH₃), 1.3-1.9 ppm (8H, -CH₂(CH₂)₄CH₃), 4.0-4.1 ppm (2H, -CH₂CH₂O-), 7.0-8.2 ppm (8H, Ar : aromatic ring of azobenzene), 13.1-13.2 ppm (1H, Ar-COOH).

C₁₂ₐzo-OH (4-(4-dodecyloxyphenylazo)benzoic acid): ¹H NMR (DMSO-d₆, TMS) 0.8-1.0 ppm (3H, -CH₃), 1.3-1.9 ppm (20H, -CH₂(CH₂)₁₀CH₃), 4.0-4.1 ppm (2H, -CH₂CH₂O-), 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, C₁₂Azo-(GPO)₅ (m=3-10) (see Figures S1 and S2)

¹H 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).

¹H 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 $3.0 \text{ [M+H]}^+ / 1562.7 \text{ [M+H]}^+$Theory.
$^1$H NMR (D$_2$O, 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 $1831.0 \text{ [M+H]}^+ / 1829.9 \text{ [M+H]}^+$Theory.
$^1$H NMR (D$_2$O, 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 $2098.9 \text{ [M+H]}^+ / 2097.2 \text{ [M+H]}^+$Theory.
$^1$H NMR (D$_2$O, 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 $2365.4 \text{ [M+H]}^+ / 2364.5 \text{ [M+H]}^+$Theory.
$^1$H NMR (D$_2$O, 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 $2632.5 \text{ [M+H]}^+ / 2631.8 \text{ [M+H]}^+$Theory.
$^1$H NMR (D$_2$O, 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 $2900.1 \text{ [M+H]}^+ / 2899.1 \text{ [M+H]}^+$Theory.
$^1$H NMR (D$_2$O, 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 $1708.7 \text{ [M+H]}^+ / 1707.8 \text{ [M+H]}^+$Theory.
$^1$H NMR (D$_2$O, 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).
**C₆Azo-(GPO)₅**: MALDI-TOFMS 1662.9 [M+H]⁺/1662.8 [M+H]⁺Theory.

¹H NMR (D₂O/TFE, DSS) 0.8-1.0 ppm (3H, CH₃- : alkyl chain), 1.2-1.6 ppm (8H, CH₃(CH₂)₄CH₂- : 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).

**C₁₂Azo-(GPO)₅**: MALDI-TOFMS 1747.6 [M+H]⁺/1746.9 [M+H]⁺Theory.

¹H NMR (D₂O/TFE, DSS) 0.8-1.0 ppm (3H, CH₃- : alkyl chain), 1.2-1.6 ppm (20H, CH₃(CH₂)₁₀CH₂- : 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 \textbf{Azo-}(GPO)\textsubscript{n} \((n=3 \text{ (a), 4 (b), 5 (c), 6 (d), 7 (e), 8 (f), 9 (g), and 10 (h))}, \textbf{Azo-deg-}(GPO)\textsubscript{5} \textbf{(i) and C}_{m}\textbf{Azo-}(GPO)\textsubscript{5} \textbf{(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.