Electronic Supplementary Information

Amphiphilic nanogel of enzymatically synthesized glycogen as an artificial molecular chaperone for effective protein refolding

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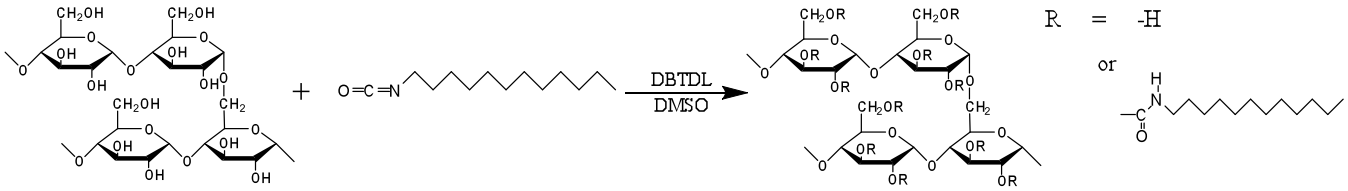
1) Syntheses of $C_{12}ESG$



Scheme S1. Synthesis of $C_{12}ESG$.

$C_{12}ESG_{0.9}$: Dodecyl isocyanate was introduced to the hydroxyl group of ESG. ESG (0.136 μmol) was dissolved in 15.0 ml of dry dimethylsulfoxide (DMSO). Then, dodecyl isocynate (18.5 μmol) and dibutyltin dilaurate as a catalyst were added to the solution under nitrogen gas at 45°C. After stirring for 24 h, the reaction mixture was poured into a concentration containing excess ethanol/diethylether (v/v: 9/1). The resulting precipitates were obtained, washed with ethanol/diethylether (v/v: 9/1), and dried overnight under a vacuum. The crude products were dissolved in DMSO, dialyzed with distilled water (molecular weight cutoff = 3,500; Spectrum Laboratories), and lyophilized. The product was analyzed by ^{1}H-nuclear magnetic resonance (600 MHz; solvent, DMSO-d_6/D_2O 9/1 (v/v), δ): 0.80—0.95 (3H, dodecyl group), 1.10—1.30 (20H, dodecyl group), 1.35—1.45 (2H, dodecyl group), 4.60—5.40 (glucose 1H [anomeric proton]). The degree of substitution was determined to be 0.9 per 100 anhydrous glucoside units.

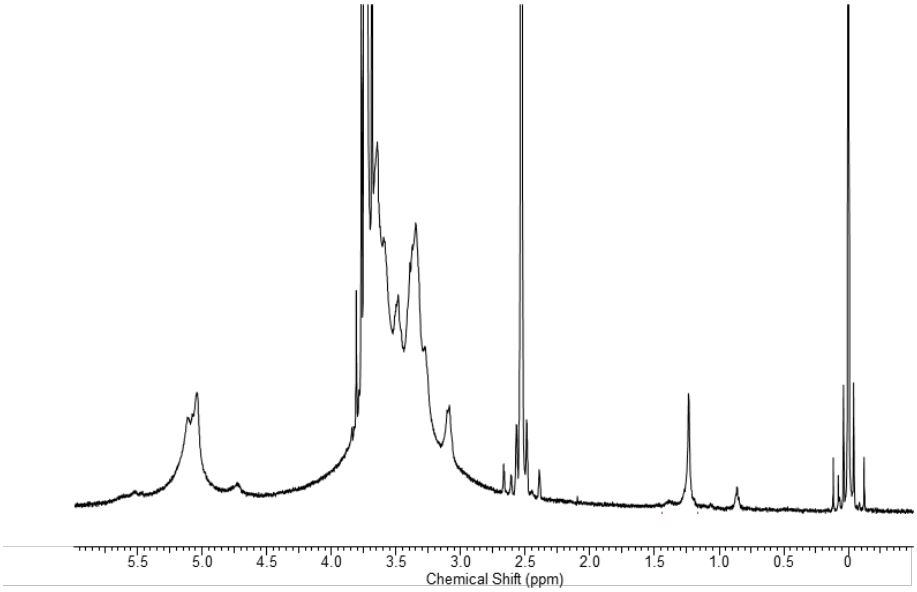


Figure S1. ^{1}H-NMR spectra of $C_{12}ESG_{0.9}$ in DMSO-d_6/D_2O 9/1 (v/v).

$C_{12}ESG_{1.3}$: Dodecyl isocyanate was introduced to the hydroxyl group of ESG. ESG (0.136 μmol) was dissolved in 15.0 ml of dry dimethylsulfoxide (DMSO). Then, dodecyl isocynate (29.6 μmol) and dibutyltin dilaurate as a catalyst were added to the solution under nitrogen gas at 45°C. After stirring for 24 h, the reaction mixture was poured into a concentration containing excess ethanol/diethylether (v/v: 9/1). The resulting precipitates were obtained, washed with ethanol/diethylether (v/v: 9/1), and dried overnight under a vacuum. The crude products were dissolved in DMSO, dialyzed with distilled water (molecular weight cutoff = 3,500; Spectrum Laboratories), and lyophilized. The product was analyzed by ^{1}H-nuclear magnetic resonance (600 MHz; solvent, DMSO-d_6/D_2O 9/1 (v/v), δ): 0.80—0.95 (3H, dodecyl group), 1.10—1.30 (20H, dodecyl group), 1.35—1.45 (2H, dodecyl group),

4.60—5.40 (glucose 1H [anomeric proton]). The degree of substitution was determined to be 1.3 per 100 anhydrous glucoside units.

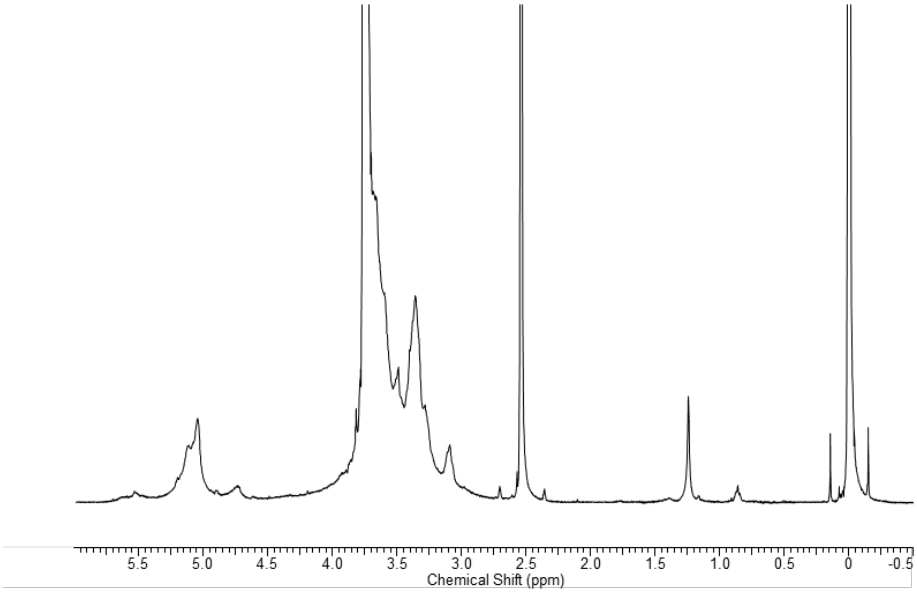


Figure S2. ^{1}H-NMR spectra of $C_{12}ESG_{1.3}$ in DMSO-d_6/D_2O 9/1 (v/v).

$C_{12}ESG_{2.0}$: Dodecyl isocyanate was introduced to the hydroxyl group of ESG. ESG (0.136 μmol) was dissolved in 15.0 ml of dry dimethylsulfoxide (DMSO). Then, dodecyl isocynate (27.8 μmol) and dibutyltin dilaurate as a catalyst were added to the solution under nitrogen gas at 45°C. After stirring for 24 h, the reaction mixture was poured into a concentration containing excess ethanol/diethylether (v/v: 9/1). The resulting precipitates were obtained, washed with ethanol/diethylether (v/v: 9/1), and dried overnight under a vacuum. The crude products were dissolved in DMSO, dialyzed with distilled water (molecular weight cutoff = 3,500; Spectrum Laboratories), and lyophilized. The product was analyzed by ^{1}H-nuclear magnetic resonance (600 MHz; solvent, DMSO-d_6/D_2O 9/1 (v/v), δ): 0.80—0.95 (3H, dodecyl group), 1.10—1.30 (20H, dodecyl group), 1.35—1.45 (2H, dodecyl group), 4.60—5.40 (glucose 1H [anomeric proton]). The degree of substitution was determined to be 2.0 per 100 anhydrous glucoside units.

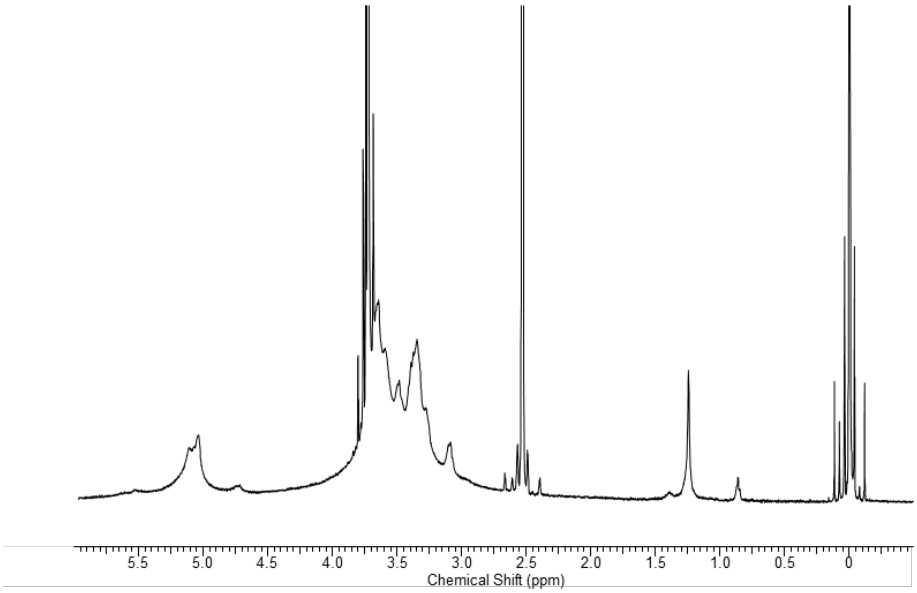


Figure S3. ^{1}H-NMR spectra of $C_{12}ESG_{2.0}$ in DMSO-d_6/D_2O 9/1 (v/v).

$C_{12}ESG_{2.8}$: Dodecyl isocyanate was introduced to the hydroxyl group of ESG. ESG (0.136 μmol) was dissolved in 15.0 ml of dry dimethylsulfoxide (DMSO). Then, dodecyl isocynate (46.3 μmol) and dibutyltin dilaurate as a catalyst were added to the solution under nitrogen gas at 45°C. After stirring for 24 h, the reaction mixture was poured into a concentration containing excess ethanol/diethylether (v/v: 9/1). The resulting precipitates were obtained, washed with ethanol/diethylether (v/v: 9/1), and dried overnight under a vacuum. The crude products were dissolved in DMSO, dialyzed with distilled water (molecular weight cutoff = 3,500; Spectrum Laboratories), and lyophilized. The product was analyzed by ^{1}H-nuclear magnetic resonance (600 MHz; solvent, DMSO-d_6/D_2O 9/1 (v/v), δ): 0.80—0.95 (3H, dodecyl group), 1.10—1.30 (20H, dodecyl group), 1.35—1.45 (2H, dodecyl group), 4.60—5.40 (glucose 1H [anomeric proton]). The degree of substitution was determined to be 2.8 per 100 anhydrous glucoside units.

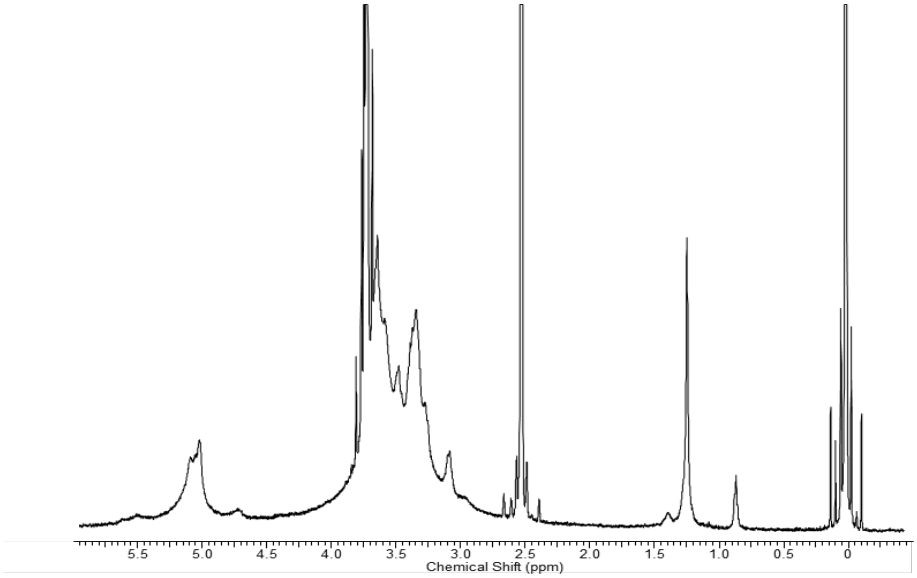
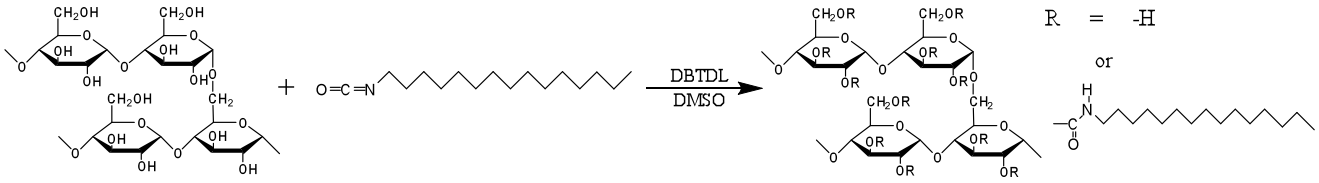


Figure S4. ^{1}H-NMR spectra of $C_{12}ESG_{2.8}$ in DMSO-d_6/D_2O 9/1 (v/v).

2) Synthesis of C_{15}ESG



Scheme S5. Synthesis of C_{15}ESG.

$C_{15}ESG_{1.9}$: Pentadecyl isocyanate was introduced to the hydroxyl group of ESG. ESG (0.682 μmol) was dissolved in 15.0 ml of dry dimethylsulfoxide (DMSO). Then, pentadecyl isocynate (11.1 μmol) and dibutyltin dilaurate as a catalyst were added to the solution under nitrogen gas at 45°C. After stirring for 24 h, the reaction mixture was poured into a concentration containing excess ethanol/diethylether (v/v: 9/1). The resulting precipitates were obtained, washed with ethanol/diethylether (v/v: 9/1), and dried overnight under a vacuum. The crude products were dissolved in DMSO, dialyzed with distilled water (molecular weight cutoff = 3,500; Spectrum Laboratories), and lyophilized. The product was analyzed by ^{1}H-nuclear magnetic resonance (600 MHz; solvent, DMSO-d_6/D_2O 9/1 (v/v), δ): 0.80—0.95 (3H, pentadecyl group), 1.10—1.30 (20H, pentadecyl group), 1.35—1.45 (2H, pentadecyl group), 4.60—5.40 (glucose 1H [anomeric proton]). The degree of substitution was determined to be 1.9 per 100 anhydrous glucoside units.

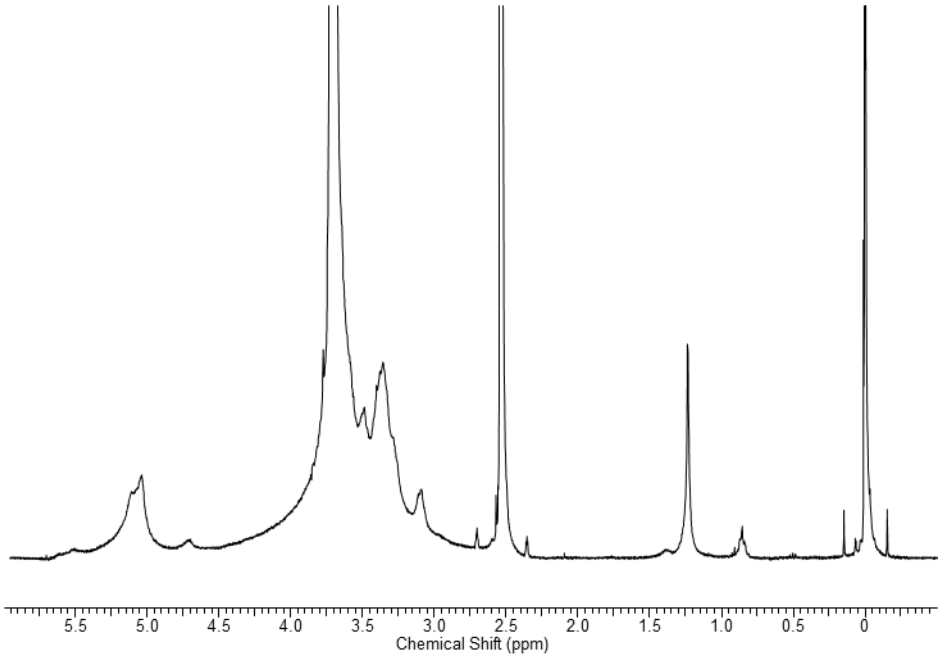
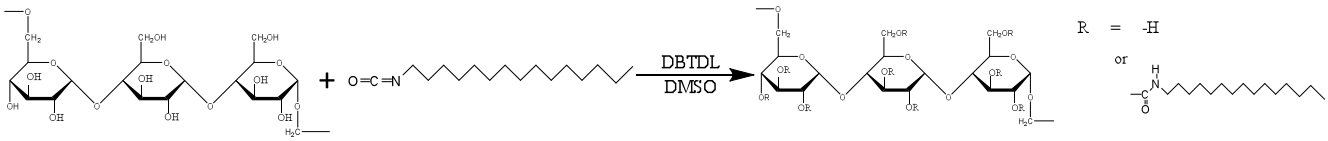


Figure S5. ^{1}H-NMR spectra of $C_{15}ESG_{1.9}$ in DMSO-d_6/D_2O 9/1 (v/v).

3) Synthesis of $C_{15}P_{1.0}$



Scheme S6. Synthesis of $C_{15}P$.

$C_{15}P_{1.0}$: Pentadecyl isocyanate was introduced to the hydroxyl group of pullulan. Pullulan (1.00 μmol) was dissolved in 50.0 ml of dry dimethylsulfoxide (DMSO). Then, pentadecyl isocynate (43.2 μmol) and dibutyltin dilaurate as a catalyst were added to the solution under nitrogen gas at 45°C. After stirring for 24 h, the reaction mixture was poured into a concentration containing excess ethanol/diethylether (v/v: 9/1). The resulting precipitates were obtained, washed with ethanol/diethylether (v/v: 9/1), and dried overnight under a vacuum. The crude products were dissolved in DMSO, dialyzed with distilled water (molecular weight cutoff = 3,500; Spectrum Laboratories), and lyophilized. The product was analyzed by ^{1}H-nuclear magnetic resonance (600 MHz; solvent, DMSO-d_6/D_2O 9/1 (v/v), δ): 0.80—0.95 (3H, pentadecyl group), 1.10—1.30 (20H, pentadecyl group), 1.35—1.45 (2H, pentadecyl group), 4.60—5.40 (glucose 1H [anomeric proton]).

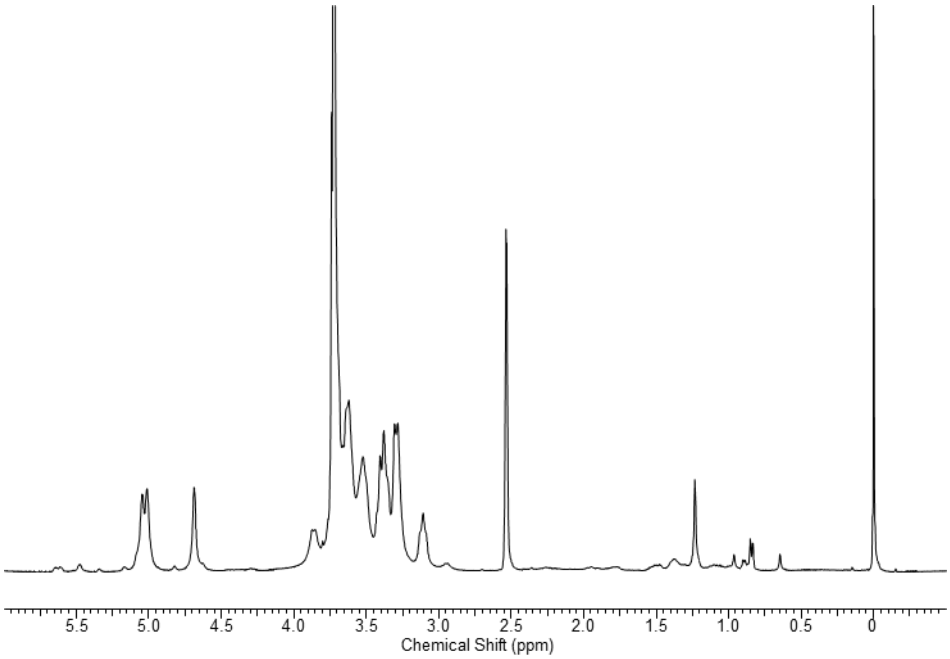


Figure S6. ^{1}H-NMR spectra of $C_{15}ESG_{1.9}$ in DMSO-d_6/D_2O 9/1 (v/v).

4) Fluorescence spectroscopic measurement

We evaluated the intensities of the bands I_1 at 374 nm and I_3 at 385 nm, and plotted the I_1 to I_3 ratio versus the C_{12}ESG concentration (Figure S2). The solutions for analysis were prepared as follows. The stock solution (15 μL) of pyrene in ethanol (1×10^{-4} M) was placed in several empty vials. The ethanol was evaporated by a gentle stream of gaseous nitrogen to form a thin film on a bottom of the vials. The C_{12}ESG nanogel solutions (1.5 mL) at a concentration of 20.0 mg/mL were added to the vials, giving a final concentration of Py of 1×10^{-6} M in each vial. The pyrene aqueous solution (10 mL, final concentration, 1×10^{-6} M) was also prepared by the addition of PBS instead of nanogel solutions. The solutions were stirred at 25°C for 24 h under a light-protected condition. The emission spectra of pyrene in the various concentrations (0.001–20.0 mg/mL) of C_{12}ESG solutions were measured by gradually dilution by the addition of pyrene aqueous solution. The concentration of pyrene was kept at 1×10^{-6} M. The fluorescence spectra (wavelength 350–500 nm) were recorded on a fluorescence spectrophotometer (FP-6500, JASCO Co., Tokyo, Japan) using the following conditions: excitation at 339 nm, slit width 5 nm for excitation, and 5 nm for emission. The intensities of the bands I_1 at 374 nm and I_3 at 385 nm were then evaluated, and their ratio was plotted versus the concentration of C_{12}ESG.

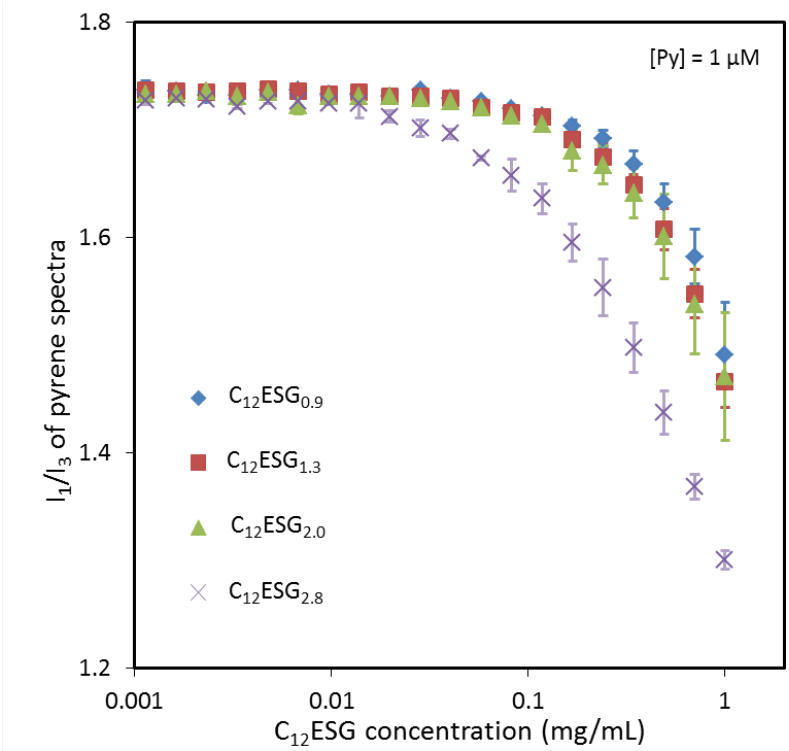


Figure S7. I_1/I_3 of pyrene spectra in the presence of $C_{12}ESG_{0.9}$, $C_{12}ESG_{1.3}$, $C_{12}ESG_{2.0}$, and $C_{12}ESG_{2.8}$.

5) Enzymatic activity assay

The recovery of the enzyme activity of chemical-denatured carbonic anhydrase (CA) was determined by a *p*-nitrophenyl acetate (*p*NPA) hydrolysis assay. A 50 μL portion of a dry acetonitrile solution of 50 mM *p*NPA was added to 90 μL of the sample solution with 360 μL of 50 mM Tris-sulfate buffer (pH 7.5). After mixing for 5 s, an increase in the *p*-nitrophenolate concentration was monitored by the absorbance at 400 nm as a function of time using a V-660 spectrophotometer (JASCO Co., Tokyo, Japan). The percent recovery of enzyme activity was calculated on the basis of the initial activity of native CA. The protein concentration of native CA was determined by absorbance at 280 nm with a coefficient of 1.83 mg protein/mL/cm.

6) Recovery of enzymatic activity of carbonic anhydrase with C15ESG and C15P

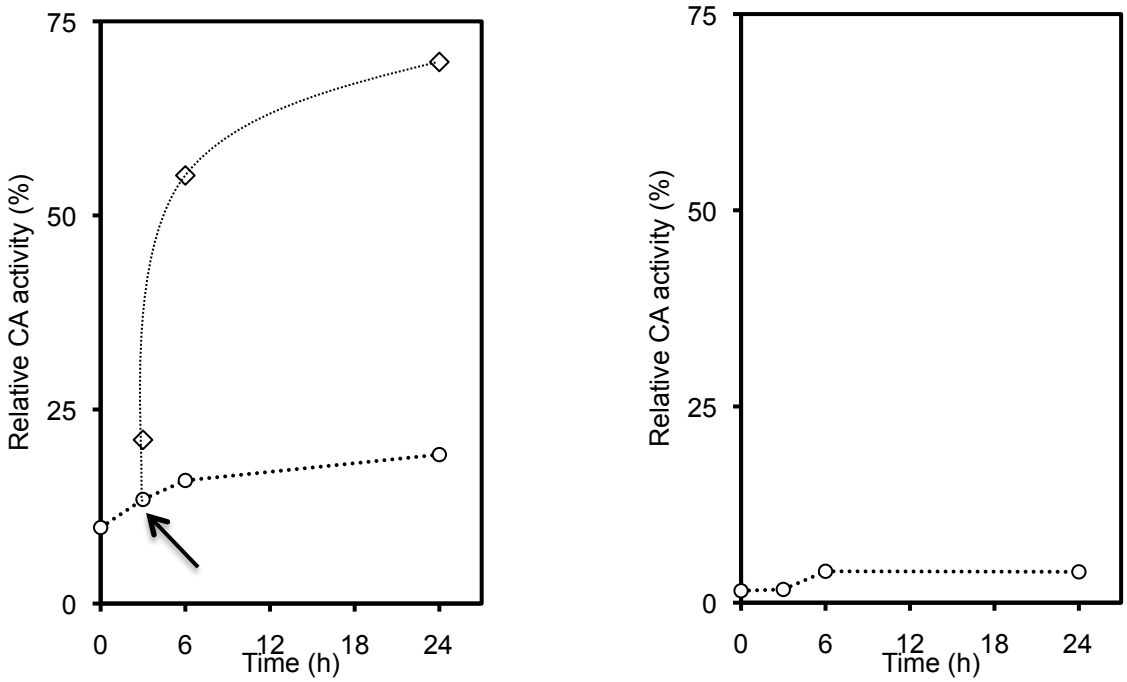


Figure S8 (Left) Time-course of refolding of chemically denatured CA (1.0 μM) after the addition of 9.1 μM of $C_{15}ESG_{1.9}$ solution (circles), and after the addition of 4 mM α-cyclodextrin to the complex (diamonds). (Right) Time-course of refolding of chemically denatured CA (1.0 μM) after the addition of 200 μM of $C_{15}P_{1.0}$ solution (circles).