Supporting Information

Molecularly Engineered "Janus GroEL": Application to Supramolecular Copolymerization with a Higher Level of Sequence Control

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1. Methods

1.1. General Methods. Unless otherwise noted, all commercial reagents were used as received from TCI, Sigma Aldrich, Wako Chemicals, and Nacalai Tesque. All DNA strands were purchased from Eurofins Genomics, which were used without further purification. Electrophoresis was performed on a 10% denaturing polyacrylamide gels (10 × 10 cm mini-gel, vertical slab purchased from Nihon Eido Co., Ltd., Japan). Unless otherwise noted, samples were dissolved in a Tris-HCl (50 mM, pH 7.6) buffer containing KCl (100 mM). Transmission electron microscopy (TEM) was performed via a JEOL type JEM-1400 transmission electron microscope operating at an accelerating voltage of 120 kV. The electron microscope specimen grid coated with a thin carbon support film was pretreated with a hydrophilizer (1.5 min) before use. Samples were dropped on the grid and removed after 1 min. The resulting sample grids were washed with milli-Q and negatively stained with a 2% aqueous solution of uranyl acetate. Cryogenic transmission electron microscopy (Cryo-TEM) was performed using a JEOL 2100F operated at an accelerating voltage of 200 kV with a Gatan 914 Cryo Transfer Holder. Samples were prepared by the ice-embedding method. Briefly, samples were put onto a hydrophilized copper microgrid in an environmental chamber with a relative humidity of 90% at a temperature of 24 °C using a Leica EM GP plunge freezer. After removing excess liquid with filter paper, the grid was plunge-frozen into liquid ethane (-175 °C) and then loaded on a specimen holder at the liquid nitrogen temperature. Small-angle X-ray scattering (SAXS) measurements were carried out by using a Rigaku model NANOPIX 3.5m system with a Rigaku model HyPix-6000 detector. The scattering vector q ($q = 4\pi\sin\theta/\lambda$; 2θ and λ = scattering angle and wavelength of an incident X-ray beam [1.54 Å], respectively) and position of an incident X-ray beam on the detector were calibrated using several orders of layer diffractions from silver behenate (d = 58.380 Å). The sample-to-detector distance was 1.34 m, where acquired scattering 2D images were integrated along the Debye–Scherrer ring by using a Rigaku 2DP software, affording the corresponding one-dimensional profiles. Samples held in a glass capillary (1.5 mm in diameter) were measured at a constant temperature using a temperature controller. Analytical size exclusion chromatography (SEC) was performed at room temperature with a JASCO type PU-2080i$_{plus}$ high performance liquid chromatograph (HPLC) equipped with a JASCO type UV-2077$_{plus}$ variable wavelength UV-vis detector. A 4.6 mm-φ × 300 mm column (Shodex KW405-4F) was used with

an elution buffer (50 mM Tris-HCl, 100 mM KCl) with a flow rate of 0.3 mL min^{-1}. To purify samples, ultrafiltration was performed with centrifugal filters with cut-off molecular weights of 3K and 100K (Amicon® Ultra-0.5 mL 3K and 100K). UV absorbance was measured using a Thermo Scientific NanoDrop ND-2000c spectrophotometer. GroEL concentrations were measured by the absorbance at 280 nm using the known extinction coefficient of GroEL (ε_{280} = 130480 M^{-1} cm^{-1}). DNA concentrations were calculated from the absorbance at 260 nm using the estimated extinction coefficients (https://sg.idtdna.com/calc/analyzer). A GeneQ thermal cycler (Hangzhou Bioer Technology Co., LTD.) was used for the protein assembly.

1.2. Preparation of DNA-Appended Molecular Chaperones. DNA conjugation was performed following the previous procedure. [S1] To DNA solutions dissolved in HKM buffer (25 mM HEPES-KOH, 100 mM KCl, 5 mM $MgCl_2$, pH 8.0) was added 100 equivalents of 3-Maleimidopropionic acid N-hydroxysuccinimide ester and the reaction mixture was incubated for 2 h at 37 °C (see Table 1 for the DNA sequences). Then, the reaction mixture was purified thoroughly via ultrafiltration using an Amicon® Ultra-0.5 mL 3K filter for 8 times. To a ^{Cys}**GroEL**^{Cys} solution (Tris 50 mM, KCl 100 mM, pH 7.6), which was expressed and purified following the previous procedure [S1], was added 200 equivalents of TCEP to the thiols on ^{Cys}**GroEL**^{Cys} and the reaction mixture was incubated for 2 h at 37 °C. The reaction mixture was then purified by ultrafiltration using an Amicon® Ultra-0.5 mL 100K filter, 4 times to remove the excess amount of TCEP. The 2 purified solutions of the maleimide-functionalized DNA and ^{Cys}**GroEL**^{Cys} were mixed ([DNA]/[SH on GroEL] = 8) in the buffer (50 mM Tris, 100 mM KCl, 50 mM $MgCl_2$, pH 7.6) and incubated for 2 h at 37 °C. The reaction mixture was analyzed by SDS-PAGE dyed with Coomassie Brilliant Blue (CBB). The conjugation yield of DNA to GroEL was estimated from the relative intensities of the DNA-attached bands on the SDS-PAGE gel. Ultrafiltration using an Amicon® Ultra-0.5 mL 100K filter was performed 10 times to remove the unreacted DNA strands.

1.3. Synthesis and Purification of Janus ^{A}**GroEL**^{B}. To a mixture of DNA-appended GroELs (^{A}**GroEL**^{A} and ^{B}**GroEL**^{B}, 1.5 μM each in 50 mM Tris-HCl, 100 mM KCl, 20 mM $MgCl_2$, pH 7.6)

was added 5 mM ATP (total volume = 52 μL) and the mixture was incubated at 37 °C for 10 min. 100-nt DNA **A'** (30 equivalents to $^{\mathbf{A}}$**GroEL**$^{\mathbf{A}}$), which is complementary to the DNA strand **A**, was added to the mixture and incubated for 1 h at 25 °C. The mixture was analyzed by SEC and the fraction containing the first 2 peaks containing $^{\mathbf{A'/A}}$**GroEL**$^{\mathbf{A/A'}}$ and $^{\mathbf{A'/A}}$**GroEL**$^{\mathbf{B}}$ were collected. Ultrafiltration using an Amicon® Ultra-0.5 mL 100K filter was performed to concentrate the sample. To this solution was added 2.8 nmol of **A''**, which was designed to liberate **A'** on GroEL via toehold-mediated DNA strand displacement, and 1.2 nmol of a 60-nt complementary DNA to **B**, **B'**. The mixture was incubated at 25 °C for 1 h. The fractions containing $^{\mathbf{A}}$**GroEL**$^{\mathbf{B/B'}}$ were collected by SEC. Analogous to the previous step, ultrafiltration using an Amicon® Ultra-0.5 mL 100K filter was performed. 1.4 nmol of **B''**, which was designed to liberate **B'** on GroEL via toehold-mediated DNA strand displacement, was added to the solution and incubated for 1 h at 25 °C. SEC was performed to collect the fractions containing $^{\mathbf{A}}$**GroEL**$^{\mathbf{B}}$.

2. Supplementary Figures

2.1. SDS-PAGE of ^{A}**GroEL**^{A} and ^{B}**GroEL**^{B}

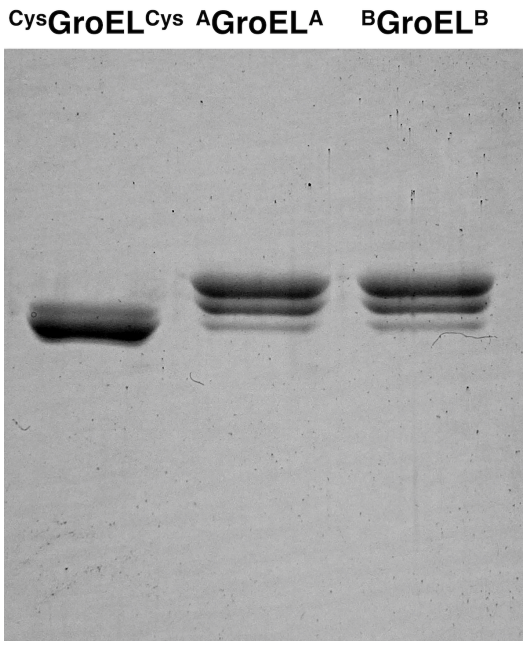

Figure S1. SDS-PAGE profile of ^{cys}**GroEL**^{cys} and DNA-appended GroELs, ^{A}**GroEL**^{A} and ^{B}**GroEL**^{B}, stained with CBB.

2.2. Electronic Absorption Spectrum of Janus ^{A}**GroEL**^{B}

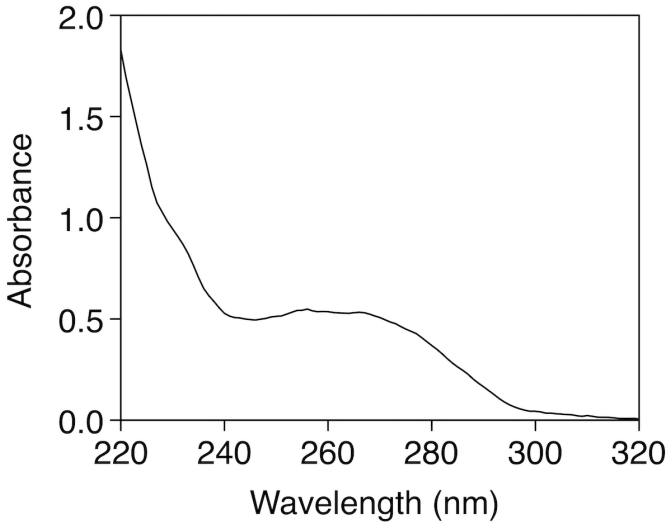

Figure S2. Representative electronic absorption spectrum of isolated Janus ^{A}**GroEL**^{B}. The concentration of ^{A}**GroEL**^{B} was calculated from the peak intensity at 260 nm using average extinction coefficients of ^{A}**GroEL**^{A} and ^{B}**GroEL**^{B}.

2.3. Transmission Electron Microscopy (TEM) Image of Janus ^{A}**GroEL**^{B}

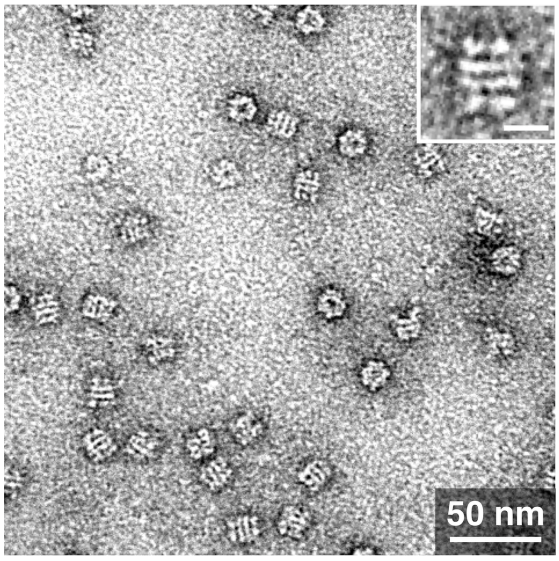

Figure S3. TEM image of Janus ^{A}**GroEL**^{B} stained with uranyl acetate (inset scale bar; 10 nm).

2.4. TEM Images of -(**$^{B*/B}$GroEL$^{A/A*/A*/A}$GroEL$^{B/B*}$**)-$_n$

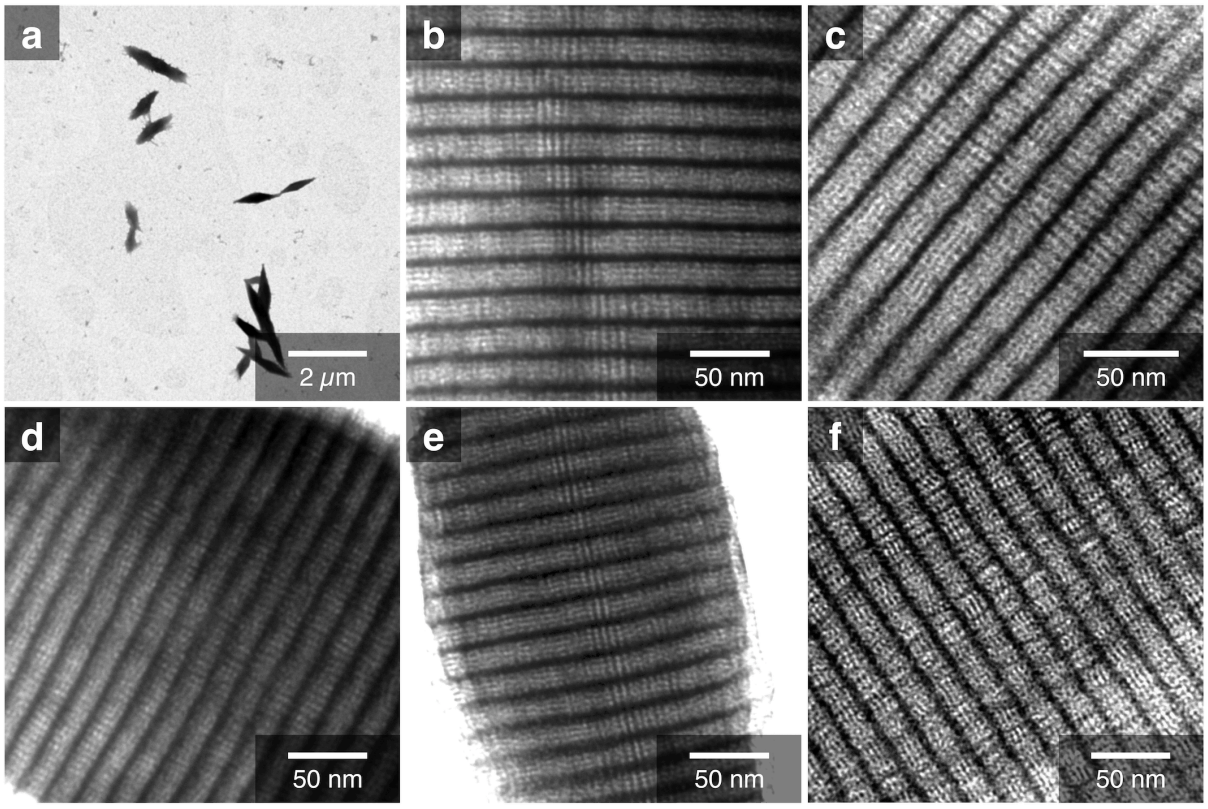

Figure S4. Ternary supramolecular copolymerization of **^{A}GroEL^{B}** (0.1 μM), **A*** (10 μM), and **B*** (10 μM) in Tris buffer (50 mM Tris-HCl, 100 mM KCl, 100 mM $MgCl_2$, pH 7.6). (a) TEM image of the assembled mixture obtained by cooling from 45 °C to 20 °C at a rate of 0.03 °C/min. (b-f) Magnified TEM images of individual assembled objects in (a).

2.5. TEM Image of -($^{A*/A}$**GroEL**$^{A/A*}$)-$_n$

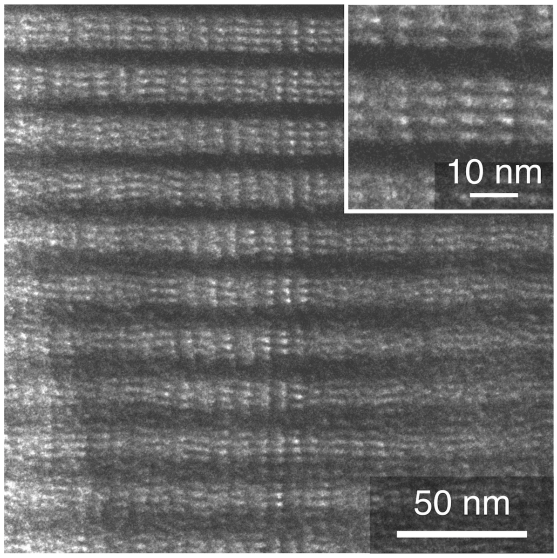

Figure S5. Supramolecular copolymerization of ^{A}**GroEL**^{A} (0.05 μM) and **A*** (5 μM) in Tris buffer (50 mM Tris-HCl, 100 mM KCl, 50 mM $MgCl_2$, pH 7.6). TEM image of the assembled mixture obtained by cooling from 45 °C to 20 °C at a rate of 0.03 °C/min. The image shows four lateral stripes in the brighter region, which is consistent with the characteristic electron microscopic feature of GroEL (Figure S3), suggesting that the protein structure is maintained in the lamellar assembly.

2.6. Cryogenic TEM (cryo-TEM) Images of -($^{A*/A}$**GroEL**$^{A/A*}$)-$_n$

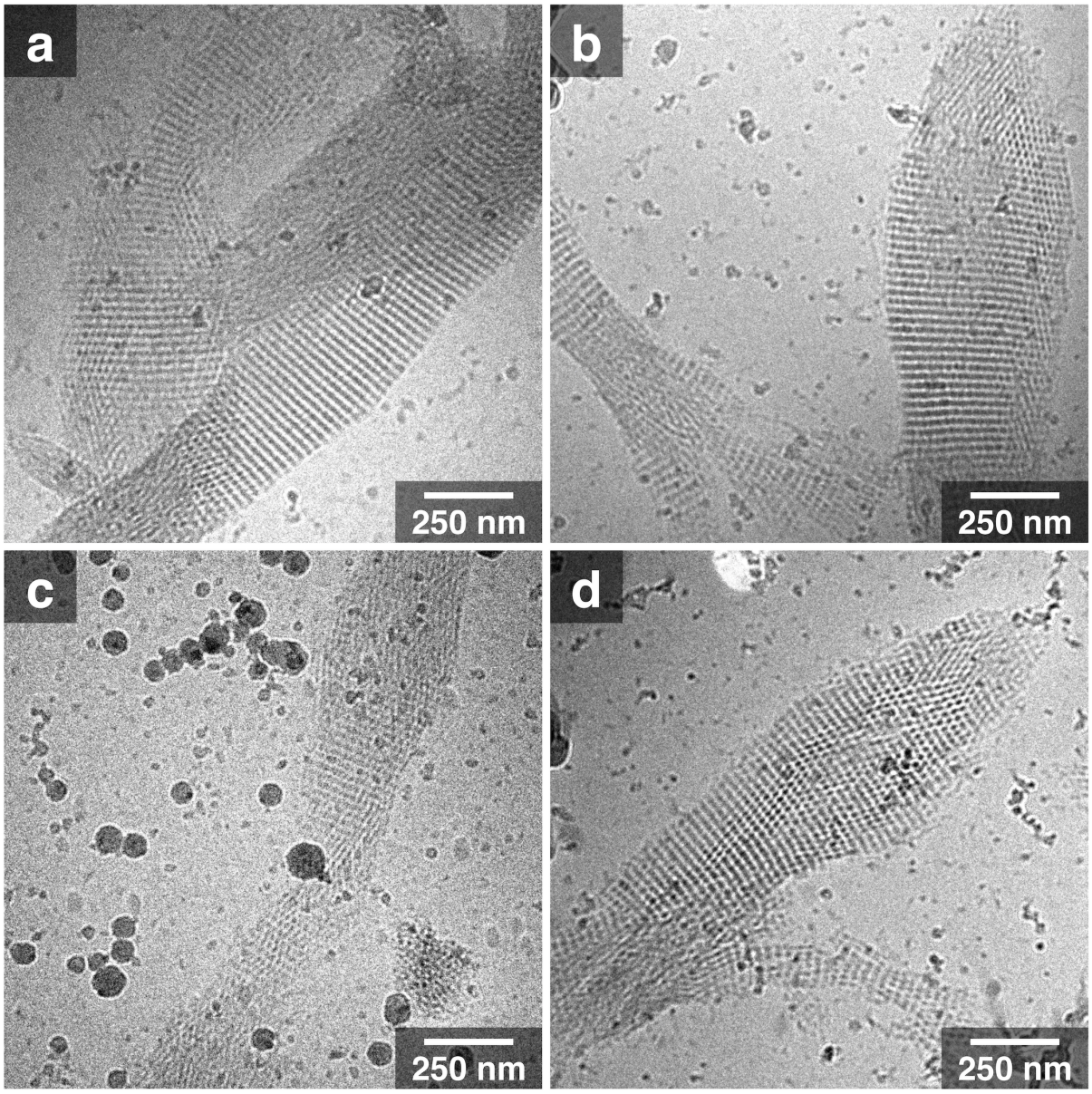

Figure S6. (a-d) Supramolecular copolymerization of ^{A}**GroEL**^{A} (0.05 μM) and **A*** (50 μM; 10 times larger than usual for obtaining smaller assemblies) in Tris buffer (50 mM Tris-HCl, 100 mM KCl, 50 mM $MgCl_2$, pH 7.6). Cryogenic transmission electron microscopy (cryo-TEM) images of the assembled mixture obtained by cooling from 45 °C to 20 °C at a rate of 0.03 °C/min. The lamellar interlayer distance was estimated as 24.0 ± 1.5 nm.

2.7. Solution Small-Angle X-Ray Scattering Profiles of -($^{A*/A}$**GroEL**$^{A/A*}$)-$_n$

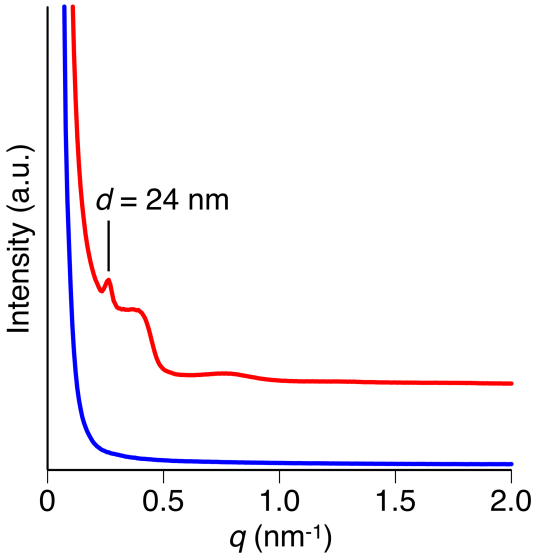

Figure S7. Supramolecular copolymerization of ^{A}**GroEL**^{A} (4 μM) and **A*** (200 μM) in Tris buffer (50 mM Tris-HCl, 100 mM KCl, 50 mM $MgCl_2$, pH 7.6). Solution small-angle X-ray scattering (SAXS) profiles of the assembled mixture obtained by cooling from 45 °C to 20 °C at a rate of 0.03 °C/min. The SAXS profiles were recorded at 20 °C (red; lamellar assembly) and 50 °C (blue; dissociated into the comonomers).

2.8. Computed Fast Fourier Transform of TEM Image of -($^{A*/A}$**GroEL**$^{A/A*}$)-$_n$

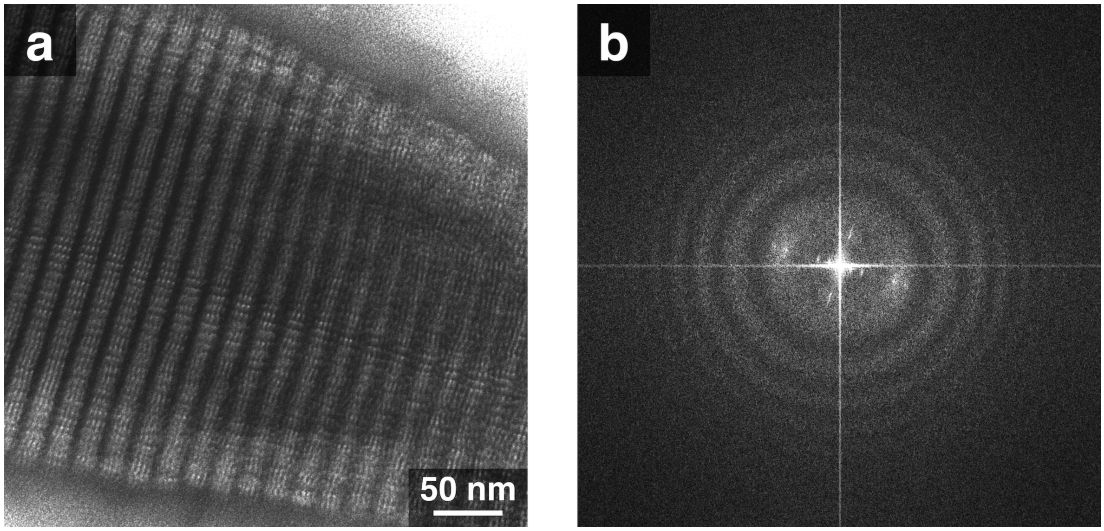

Figure S8. Supramolecular copolymerization of ^{A}**GroEL**^{A} (0.05 μM) and **A*** (5 μM) in Tris buffer (50 mM Tris-HCl, 100 mM KCl, 50 mM $MgCl_2$, pH 7.6). (a) TEM image of the assembled mixture obtained by cooling from 45 °C to 20 °C at a rate of 0.03 °C/min. (b) Fast Fourier transform (FFM) of the TEM image (a).

3. Supporting Reference

[S1] Kashiwagi, D.; Sim, S.; Niwa, T.; Taguchi, H.; Aida, T. Protein Nanotube Selectively Cleavable with DNA: Supramolecular Polymerization of "DNA-Appended Molecular Chaperones". *J. Am. Chem. Soc.* **2018,** *140,* 26−29.