Electronic Supplementary Information for

Photocontrolled Reversible Morphology Conversion of Protein Nanowires Medicated by Azobenzene-Cored Dendrimer

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1. Materials and Method

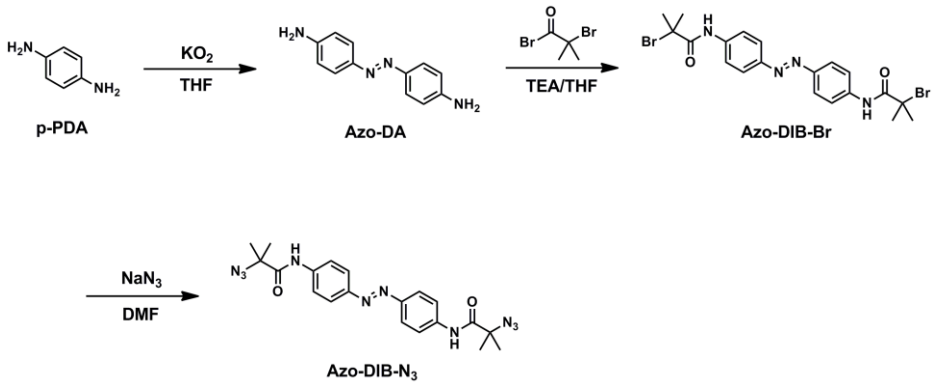
1.1 Chemicals

p-Phenylenediamine, potassium superoxide, sodium azide, copper (II) sulfate pentahydrate, L-ascorbic acid sodium salt, fluorescein isothiocyanate and all of the deuterated solvents were purchased from Sigma–Aldrich Chemical Co. 2-Bromoisobutyryl bromide, propargylamine, 1, 2-ethylenediamine, and methyl acrylate were purchased from J&K Chemical LTD. Dichloromethane, ethyl acetate and methanol were purchased from Beijing Chemical Plant. N,N-Dimethylformamide and trimethylamine were purchased from Sinopharm Chemical Reagent Co. Ltd. and dried over calcium hydride before using. Tetrahydrofuran was purchased from Sinopharm Chemical Reagent Co. Ltd. and dried with sodium.

1.2 Instruments

^{1}H NMR spectra was measured on a Bruker 510 spectrometer (500 MHz) using DMSO, $CDCl_3$ or D_2O as solvent with tetramethylsilane (TMS) as a reference. Liquid Chromatograph-Mass Spectrometer (LC-MS, Agilent1290-micrOTOF-Q II) or Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) were employed to analyze the molecule weight of samples. UV–Vis spectrums were obtained with a Shimadzu 3100 UV-VIS-NIR Recording Spectrophotometer interfaced with a personal computer. Dynamic Light Scattering (DLS) experiments were carried out with Malvern Instrument Zetasizer Nano ZS equipped with a He–Ne laser (633 nm, 4 mW) and an avalanche photodiode detector. Atomic Force Microscopy (AFM) measurements were performed on a NanoScope Multimode AFM (Veeco, USA) using the tapping mode AFM with a SiN4 tip with a radius of 10~20 nm. Transmission Electron Microscopy (TEM) was recorded on a JEM-2100F instrument with an accelerating voltage of 200 kV.

2. Synthesis of Photoresponsive Azo-DIB-N_3



2.1 Synthesis of 4,4'-Azodianiline (Azo-DA)

p-Phenylenediamine (1.08 g, 10 mmol) and potassium superoxide (2.84 g, 40 mmol) were dispersed in 100 mL of anhydrous tetrahydrofuran. The solution was degassed by high-purity nitrogen and reacted under reflux for 24 h. After filtration and solvent evaporation, the crimson 4,4'-azodianiline was purified by silica column chromatography (200–300 mesh) using dichloromethane/ethyl acetate (10:1, v/v) as the eluent (0.85 g, yield: 40%). ^{1}H-NMR (500 MHz, DMSO-d6, δ=2.50 ppm) δ=7.52 (d, 4H, -NCCH-), 6.62 (d, 4H, -CHCNH_2), 5.72 (s, 4H, -NH_2).

2.2 Synthesis of Azo-DIB-Br

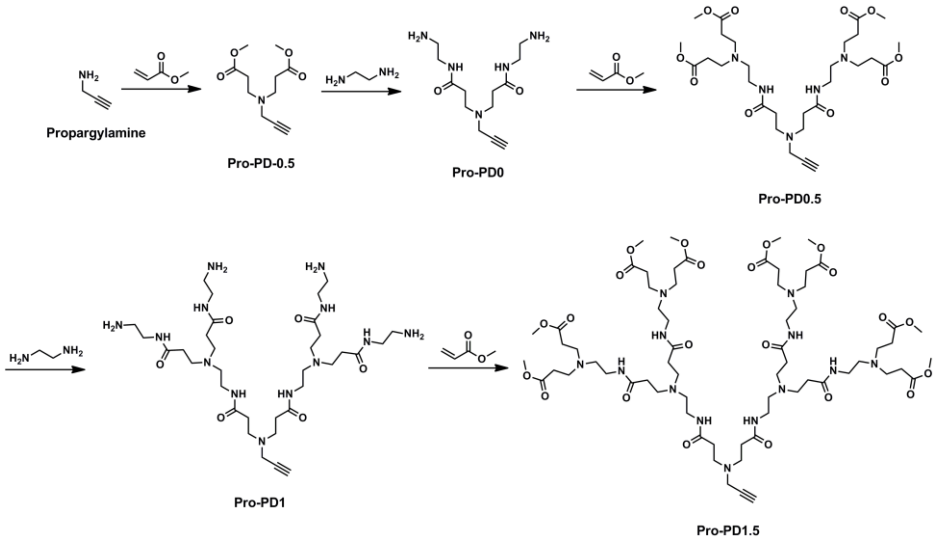
4,4'-Azodianiline (212 mg, 1.0 mmol) was dissolved in 10 mL of anhydrous tetrahydrofuran containing appropriate amount of trimethylamine (695 μL, 5.0 mmol) and stirred under ice-bath. 2-Bromoisobutyryl bromide (310 μL, 2.5 mmol) in 10 mL of anhydrous tetrahydrofuran was slowly dropwise added to the mixture within 30 min under ice-bath. The mixture was vigorously stirring for 24h at room temperature and then evaporated under reduced pressure to remove the vast majority of solvent. The residue was washed with petroleum ether and filtrated to afford yellow solid (460 mg, 91%). ^{1}H-NMR (500 MHz, DMSO-d6, δ=2.50 ppm) δ=10.13 (s, 2H, -CNHCO-), 7.91 (m, 8H, -CCHC-), 2.03 (s, 12H, -CH_3).

2.3 Synthesis of Azo-DIB-N_3

Azo-DIB-Br (460 mg, 0.91 mmol) was dissolved in 15 mL of N,N-dimethylformamide and added with sodium azide (473 mg, 7.28 mmol). The mixture was degassed by high-purity nitrogen and reacted at 60 ℃ for 16 h. After evaporating the solvent under reduced

pressure, the residue was washed with water to remove the inorganic salt. The crude product was purified by silica column chromatography (200–300 mesh) using dichloromethane/ethyl acetate (50:1, v/v) as the eluent to afford yellow solid (335 mg, yield: 85%). ^{1}H-NMR (500 MHz, DMSO-d6, δ=2.50 ppm) δ=10.03 (s, 2H, -CNHCO-), 7.90 (m, 8H, -CCHC-), 1.56 (s, 12H, -CH_3) (Figure S1). MS (ESI, m/z) Calc. for $C_{20}H_{22}N_{10}O_2$, 434.19; found: 435.2 $[M+H]^+$ (Figure S2).

3. Preparation of Propargyl-PAMAM Dendrons.



In this study, the propargyl-PAMAM dendrons were synthesized similar to our previously reported method.[1] As shown in Figure S6, propargylamine was employed as dendrimer core and reacted with methyl acrylate by *Michael addition* reaction to form double ester. Then, the double ester was aminolysized with excess of 1, 2-ethylenediamine to form double amine, as generation zero (Pro-PD0). We could get the generation 1.5th PAMAM dendrons by alternatively *Michael additions* and amination reactions.

3.1 Synthesis of Pro-PD-0.5

Propargylamine (1.6 mL, 25 mmol) was dissolved in 20 mL of anhydrous methanol. The solution was degassed by high-purity nitrogen and stirred under ice-bath for 30 min. Then, methyl acrylate (12 mL, 125 mmol) was dropwise added to the solution and kept stirring for 24 h at room temperature. The residual methyl acrylate and solvent was evaporated under reduced pressure to obtain translucent oil (5.5 g, yield: 97 %).

3.2 Synthesis of Pro-PD0

1,2-Ethylenediamine (13 mL, 200 mmol) was dissolved in 20 mL of anhydrous methanol and added dropwise to a solution of Pro-PD-0.5 (4.54 g, 20 mmol) in 30 mL of anhydrous methanol under nitrogen in an ice bath. The reaction mixture was isolated from light and kept stirring for 48 h at room temperature. Then, the mixture was evaporated under reduced pressure to remove the solvent and residual 1, 2-ethylenediamine (5.4 g, yield: 95%). ^{1}H-NMR (500 MHz, $CDCl_3$, δ=7.26 ppm) δ=7.22 (t, 2H, -CONH-), 3.41 (d, 2H, -CCH_2-), 3.27 (q, 4H, -$CONHCH_2$-), 2.81 (m, 8H, -NCH_2CCO-, -CH_2NH_2), 2.37 (t, 4H, -CH_2CONH-), 2.21 (t, 1H, CHC-).

3.3 Synthesis of Pro-PD0.5

Pro-PD0 (2.83 g, 10 mmol) was dissolved in 20 mL of anhydrous methanol. The solution was stirred under nitrogen for 30 min at room temperature. Then, 10 mL of anhydrous methanol dissolved with methyl acrylate (18 mL, 200 mmol) was dropwise added to the solution and kept stirring for 24 h at room temperature. The residual methyl acrylate and solvent was evaporated under reduced pressure. The crude product was purified by silica column chromatography (200–300 mesh) using dichloromethane/ethyl acetate (2:1, v/v) as the eluent. (5.94 g, yield: 95%). ^{1}H-NMR (500 MHz, $CDCl_3$, δ=7.26 ppm) δ=7.08 (t, 2H, -CONH-), 3.66 (s, 12H, -$COOCH_3$) 3.46 (d, 2H, -CCH_2-), 3.29 (q, 4H, -$CONHCH_2$-), 2.85 (t, 4H, -NCH_2CCO-), 2.76 (t, 8H, -NCH_2CCO-), 2.54 (t, 4H, -$CONHCCH_2N$-), 2.43 (t, 8H, -CCH_2COO-), 2.38 (t, 4H, -CCH_2CONH-), 2.19 (t, 1H, CHC-).

3.4 Synthesis of Pro-PD1

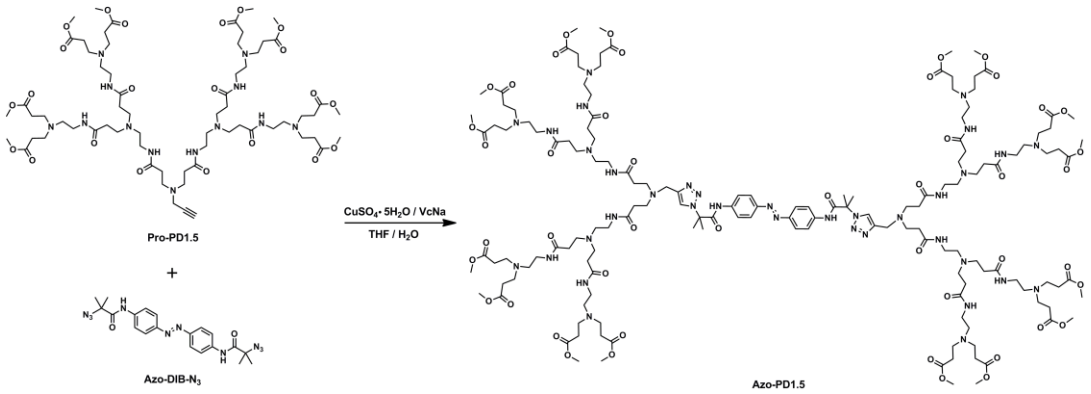
The synthesis of Pro-PD1 was very similar to that of Pro-PD0. Specifically, 1, 2-ethylenediamine (38 mL, 568 mmol) was dissolved in 20 mL of anhydrous methanol and added dropwise to a solution of Pro-PD0.5 (5.94 g, 9.5 mmol) in 20 mL of anhydrous methanol under nitrogen in an ice bath. The mixture was isolated from light and kept stirring for 48 h at room temperature. Then, the mixture was evaporated under reduced pressure to remove the solvent and residual 1, 2-ethylenediamine (6.56 g, yield: 94%). ^{1}H-NMR (500 MHz, $CDCl_3$, δ=7.26 ppm) δ=7.90 (t, 2H, -CONH-), 7.66 (t, 4H, -CONH-), 3.28 (q, 8H, -$CONHCH_2$-), 3.23 (q, 4H, -$CONHCH_2$-), 2.81 (m, 12H, -CH_2NHCO-), 2.72 (t, 8H, -CH_2NH_2), 2.51 (t, 4H, -$CONHCCH_2N$-), 2.35 (m, 12H, -CH_2CONH-), 2.22 (t, 1H, CHC-).

3.5 Synthesis of Pro-PD1.5

Pro-PD1.5 was prepared according the synthesis of Pro-PD0.5. Specifically, Pro-PD1 (3.78 g, 5.1 mmol) was dissolved in 30 mL of anhydrous methanol. The solution was stirred under nitrogen for 30 min at room temperature. Then, 30 mL of anhydrous methanol dissolved with methyl acrylate (37 mL, 410 mmol) was dropwise added to the solution and kept stirring for 48 h at room temperature. The residual methyl acrylate and solvent was evaporated under reduced pressure. The crude product was purified by silica column chromatography (200–300 mesh) using ethyl acetate/ methanol (20:1, v/v) as the eluent to afford colorless oil. (4.53 g, yield: 63%). ^{1}H-NMR (500 MHz, $CDCl_3$, δ=7.26 ppm) δ=7.80 (t, 2H, -CONH-), 7.19 (t, 4H, -CONH-), 3.66 (s, 24H, -$COOCH_3$) 3.44 (d, 2H, -CCH_2-), 3.28 (q, 12H, -$CONHCH_2$-), 2.79 (t, 12H, -NCH_2CCO-), 2.74 (t, 16H, -NCH_2CCO-), 2.60-2.50 (t, 12H, -$CONHCCH_2N$-), 2.43 (t, 16H, -CCH_2COO-), 2.37 (t, 12H, -CCH_2CONH-), 2.22 (t, 1H, CHC-) (Figure S3). MS (MALDI-TOF, m/z) Calc. for $C_{65}H_{113}N_{13}O_{22}$, 1427.81; found: 1428.8 $[M+H]^+$, 1450.8 $[M+Na]^+$, 1466.9 $[M+K]^+$ (Figure S4).

4. Synthesis of Azobenzene-cored PAMAM 4G Dendrimer

4.1 Synthesis of Azobenzene-cored PAMAM 1.5G Dendrimer *via* Click Chemistry



The azobenzene-cored PAMAM 1.5G dendrimer (AzoPD1.5) was prepared via azide-alkyne click reaction.[2] Specifically, Azo-DIB-N_3 (435 mg, 1 mmol) and Pro-PD1.5 (3.0g, 2.1 mmol) were dissolved in 20 mL of tetrahydrofuran with appropriate of copper (II) sulfate pentahydrate (25 mg, 0.1 mmol). After degassed by high-purity nitrogen, 10 mL of degassed aqueous solution containing L-ascorbic acid sodium salt (99mg, 0.5 mmol) was injected to the solution and stirred at 60 ℃ for 6 h. After evaporating the solvents under reduced

pressure, the crude product was purified by silica column chromatography (200–300 mesh) using ethyl acetate/ methanol (2:1, v/v) as the eluent to afford yellow viscous oil. (1.58 g, yield: 48 %). ^{1}H-NMR (500 MHz, $CDCl_3$, δ=7.26 ppm) δ=9.00 (s, 2H, -CNHC-), 7.82-7.68 (m, 8H, -CCHCH-), 7.80 (t, 4H, -CONH-), 7.04 (t, 8H, -CONH-), 3.81 (s, 4H, -CCH_2N-), 3.65 (s, 48H, -$COOCH_3$), 3.27 (m, 24H, -$CONHCH_2$-), 2.80 (m, 24H, -NCH_2CCO-), 2.73 (t, 32H, -NCH_2CCOO-), 2.58 (t, 8H, -$CONHCCH_2$N-), 2.52 (t, 16H, -$CONHCCH_2$N-), 2.42 (m, 32H, -CCH_2COO-), 2.35 (t, 24H, -CCH_2CONH-), 2.02 (s, 12H, -CCH_3), 1.25 (s, 2H, -NCHC-) (Figure S5). MS (ESI, m/z) Calc. for $C_{150}H_{248}N_{36}O_{46}$, 3291.79; found: 824.0 $[M+4H]^{4+}$ (Figure S6).

4.1 Synthesis of Photoisomerizable AzoPD4

The AzoPD4 dendrimer was synthesized as our previously reported. AzoPD1.5 was aminolysized with excess of 1, 2-ethylenediamine and then *Michael addition* reaction with methyl acrylate to afford AzoPD2.5 dendrimer. ^{1}H-NMR (500 MHz, $CDCl_3$, δ=7.26 ppm) δ=9.20 (s, 2H, -CNHC-), 7.90-7.64 (12H, -CONH-), (8H, -CCHCH-), 7.10 (br, 16H, -CONH-), 3.82 (s, 4H, -CCH_2N-), 3.65 (s, 96H, -$COOCH_3$), 3.27 (q, 56H, -$CONHCH_2$-), 2.80 (br, 56H, -NCH_2CCO-), 2.74 (t, 64H, -NCH_2CCOO-), 2.57 (br, 24H, -$CONHCCH_2$N-), 2.53 (t, 32H, -$CONHCCH_2$N-), 2.42 (t, 64H, -CCH_2COO-), 2.35 (br, 56H, -CCH_2CONH-), 2.02 (s, 12H, -CCH_3), 1.25 (s, 2H, -NCHC-).

Then, AzoPD2.5 dendrimer was alternatively reacted with 1, 2-ethylenediamine, methyl acrylate and 1, 2-ethylenediamine to obtain AzoPD4 dendrimer. ^{1}H-NMR (500 MHz, D_2O, δ=4.79 ppm) δ=3.80 (br, 4H, -CCH_2N-), 3.26-3.16 (br, 248H, -$CONHCH_2$-), 2.76 (br, 248H, -NCH_2CCO-), 2.68 (br, 128H, -CCH_2NH_2), 2.56 (br, 120H, -$NHCCH_2$N-), 2.36 (br, 248H, -CCH_2CONH-), 2.01 (s, 12H, -CCH_3), 1.86 (s, 2H, -NCHC-) (Figure S7).

5. Self-Assembly of AzoPD4/SP1 Nanowires

The assembly of trans-AzoPD4/SP1 nanowires was as follow: SP1 or AzoPD4 was dissolved in Milli-Q with the final concentration of 2.0 μM. Then, 100 μL of AzoPD4 was mixed with 100 μL of SP1 solution under daylight. The mixed solutions were dispersed and then let stand for 60 min before use.

Dynamic light scattering (DLS) measurements are conducted to investigate the initial assembly of AzoPD4/SP1 hybrids at room temperature using a Malvern Nano_S instrument (Malvern, U.K.). The final SP1 concentration for all samples is 1.0 μM. For AzoPD4/SP1 binary

hybrids, DLS profiles recorded the intensity in different buffers during titration of AzoPD4 into a SP1 solution.

Agarose Gel Electrophoresis: The AzoPD4/SP1 for agarose gel electrophoresis were obtained by AzoPD4 induced self-assembly of fluorescein labeled SP1 protein. First, fluorescein isothiocyanate (FITC) labeled SP1 was prepared from a solution of 2.0 mg/mL of SP1 in carbonic acid buffer solution (100 mM CBS, 125 mM NaCl, pH=9.0) and labeled with FITC solution in DMSO (0.2 mg/mL). The sample was incubated in the dark for 12 h and dialyzed with dialysis tube (Spectra/Pro Membrane, MWCO=3,500) to remove the unreacted FITC. Fluorescein labeled AzoPD4/SP1 contained 5.0 μM SP1 and different molar ratio of AzoPD4 dendrimer (between 0 μM and 50 μM) was used to agarose gel electrophoresis analysis. Agarose gels were prepared from a solution of 0.8% agarose in dilute acetate buffer (10 mM NaAc, 1.0 mM EDTA, 1.0 mM NaN_3, pH=6.3), and the samples were run at 100 V for 20 min.

6. Photocontrolled Protein Self-Assembly

The assembly of *cis*-AzoPD4/SP1 nanowires was as follow: A portable ultraviolet germicidal lamp with long wavelengths (365 nm, 8W) was served as the ultraviolet radiation source to control the isomerization process. The AzoPD4/SP1 complexes were dispersed and then incubated under ultraviolet light (365 nm) for 60 min before use. For AFM or TEM characterization, the light triggered samples were dispersed onto the freshly hydrogen-implanted (111) silicon wafer or the standard carbon-coated Formvar films on copper grids, and the samples were exposed to the ultraviolet light in the whole process.

7. Composition Analysis of Supramolecular Protein Rings

The curvature (*K*, nm^{-1}) and curvature radius (r, nm) of a curve are reciprocal relationship and are shown as follows:

$$K = r^{-1} \quad (1)$$

The closed protein rings are arranged with the diameter about 50 nm and the average protein-protein interspace angle (θ) about 10.5 º. We can calculate the curvature (*K*) of the ring and the number of SP1 proteins (N). The results showed that the *K* of the protein ring reached to 0.040 nm^{-1}, and each supramolecular protein ring composed of nearly 34 SP1 cricoid proteins.

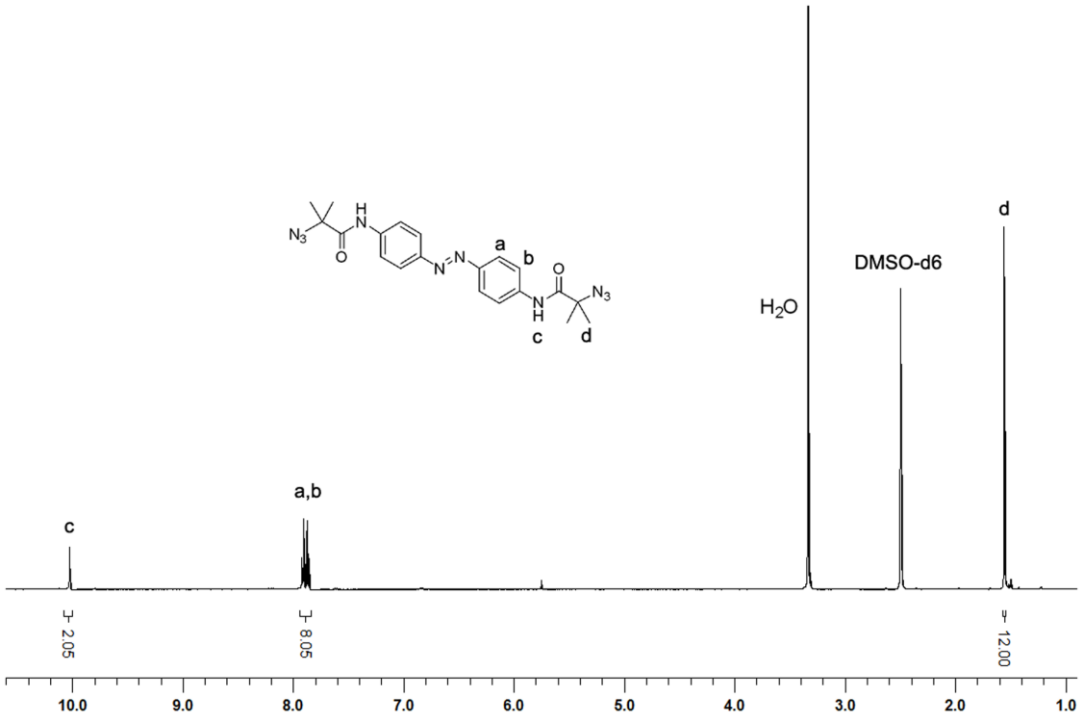


Figure S1: ^{1}H-NMR spectra of Azo-DIB-N_3.

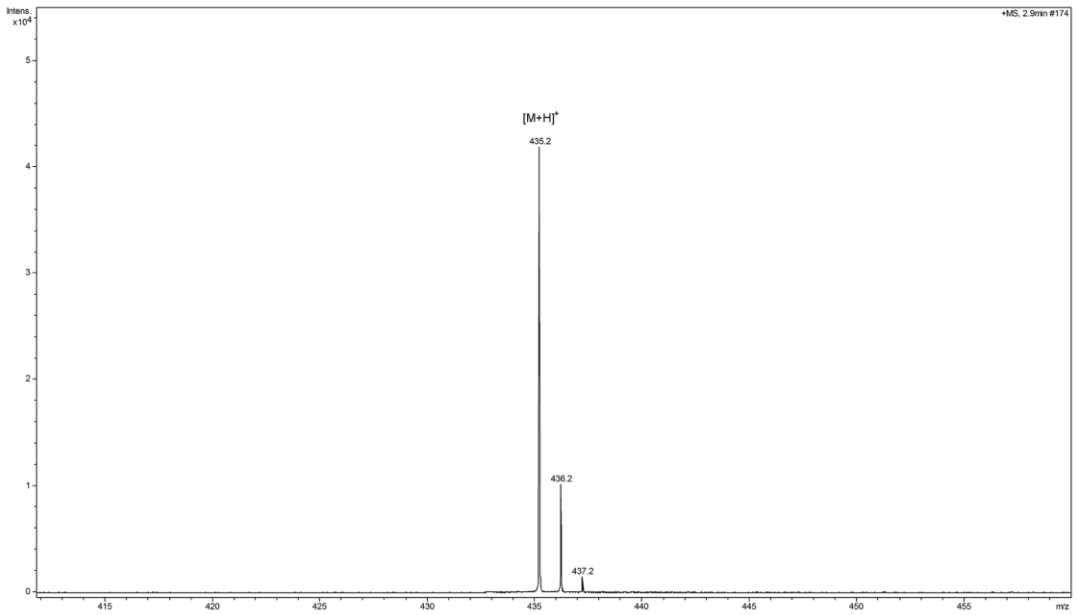


Figure S2: ESI mass spectrum of Azo-DIB-N_3.

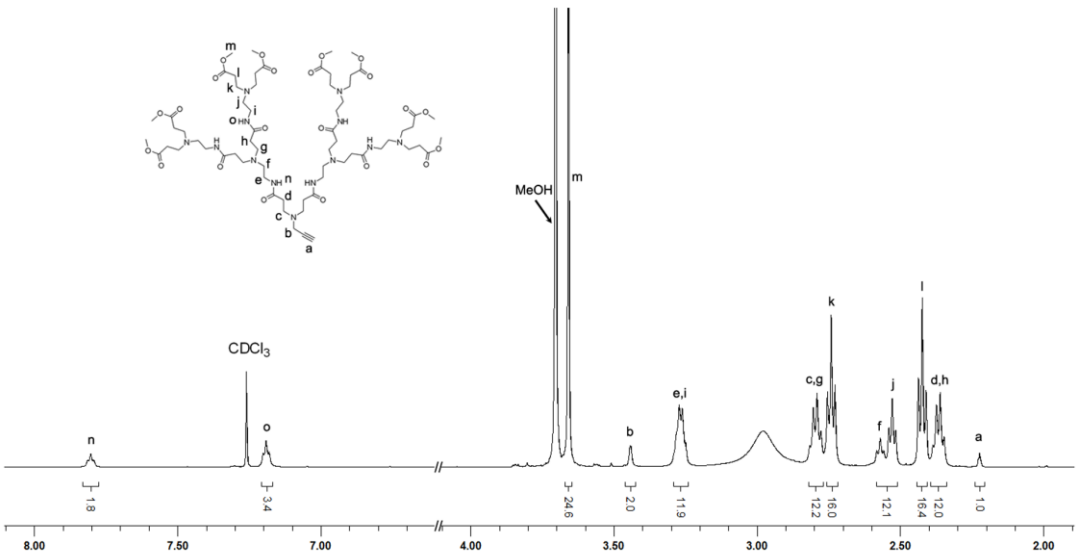


Figure S3: ^{1}H-NMR spectra of Pro-PD1.5.

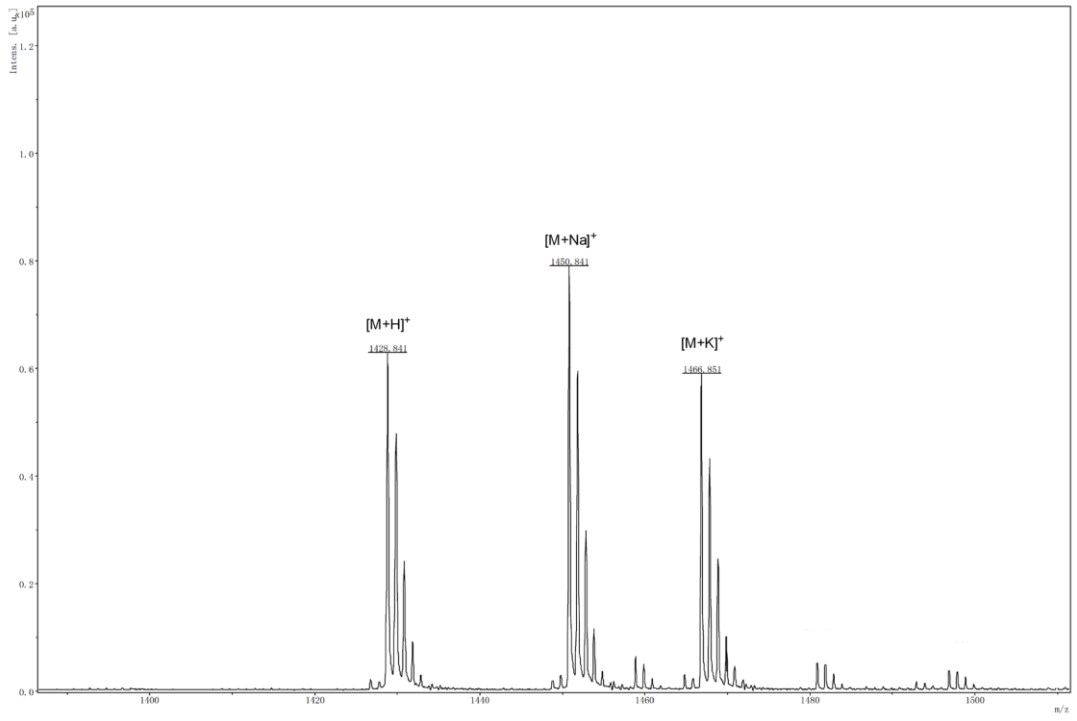


Figure S4: MALDI-TOF mass spectrum of Pro-PD1.5.

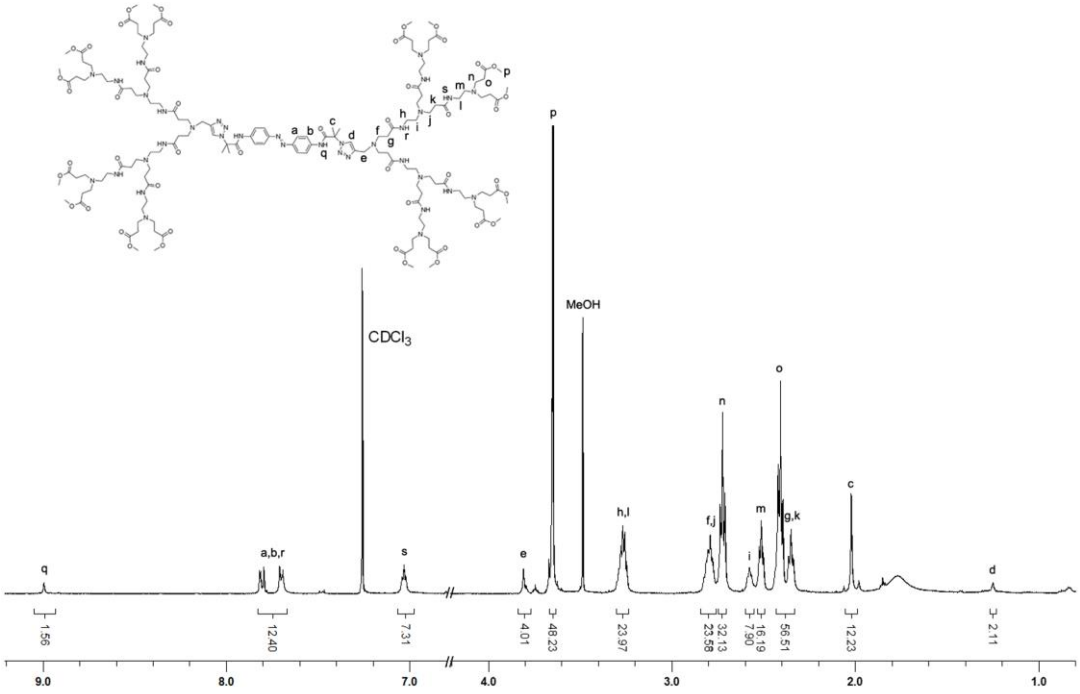


Figure S5: ^{1}H-NMR spectra of AzoPD1.5.

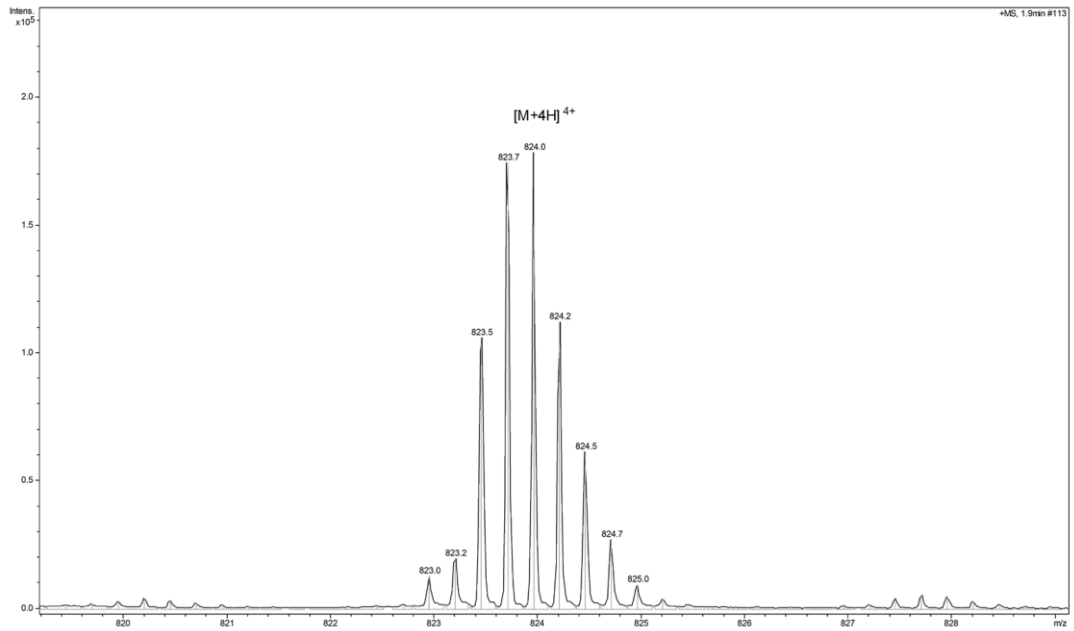


Figure S6: ESI mass spectrum of AzoPD1.5.

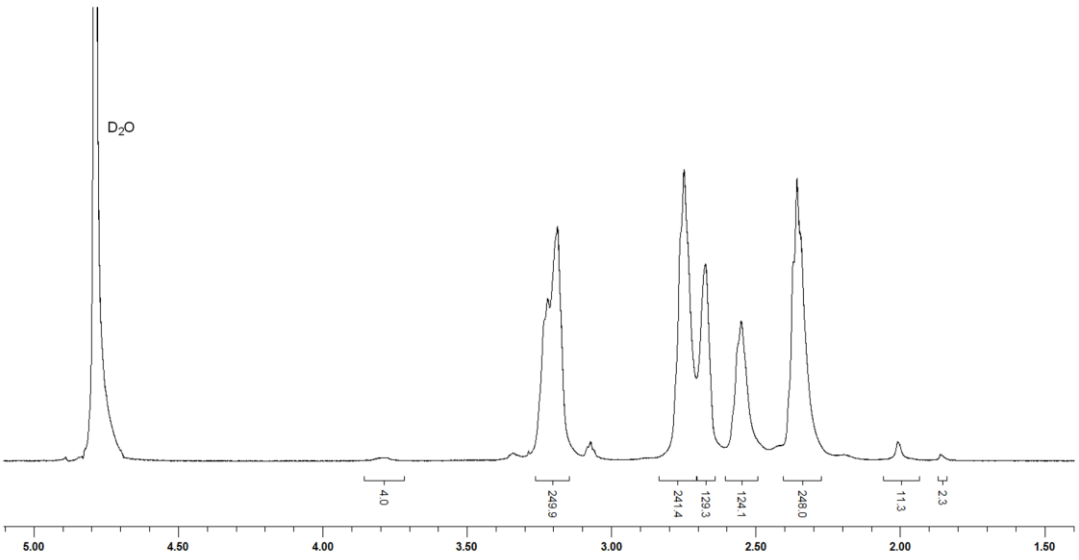


Figure S7: ^{1}H-NMR spectra of AzoPD4.

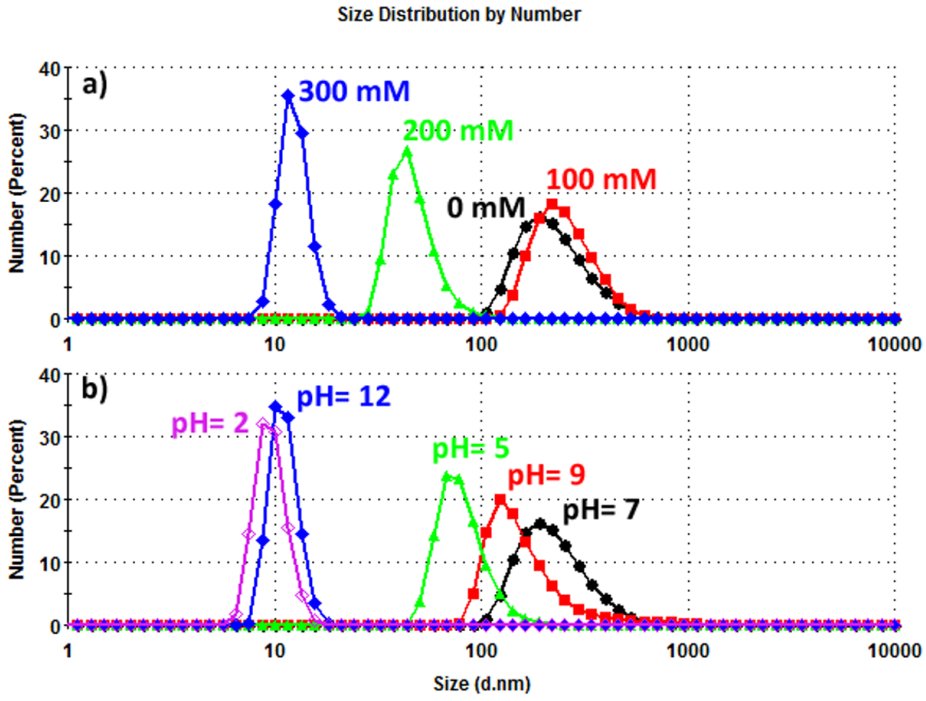


Figure S8: Stability investigation of the assembly using the dynamic light scattering (DLS) analysis. (a) The effect of ionic strengths in 0 mM (black), 100 mM (red), 200 mM (green) and 300 mM (blue) NaCl concentration; (b) The effect of pH at pH=2 (purple), 5 (green), 9 (red), and 12 (blue).

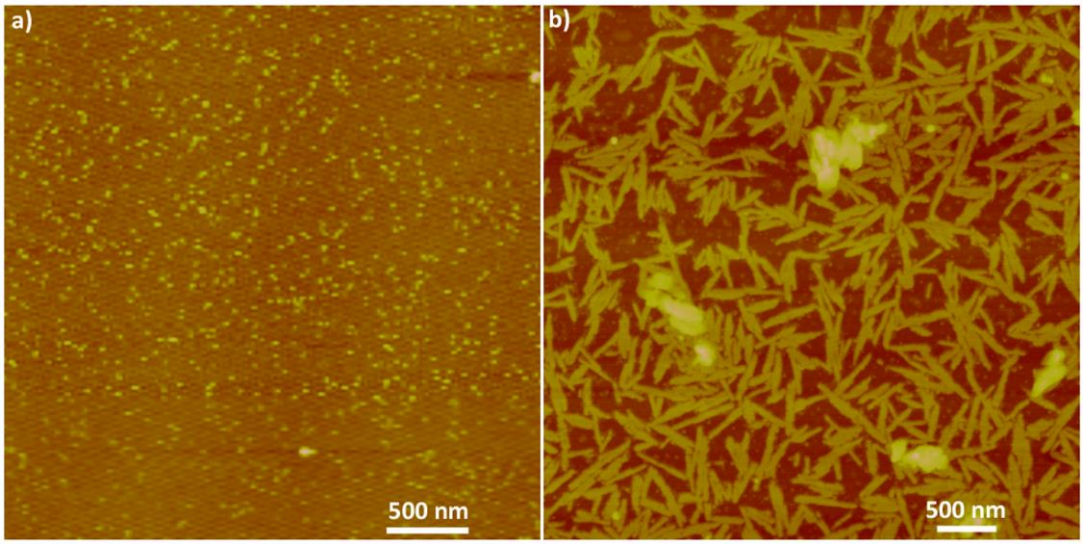


Figure S9: (a) Large scale AFM images of free SP1 (a) and AzoPD4 induced SP1 nanowires (b) under visible light.

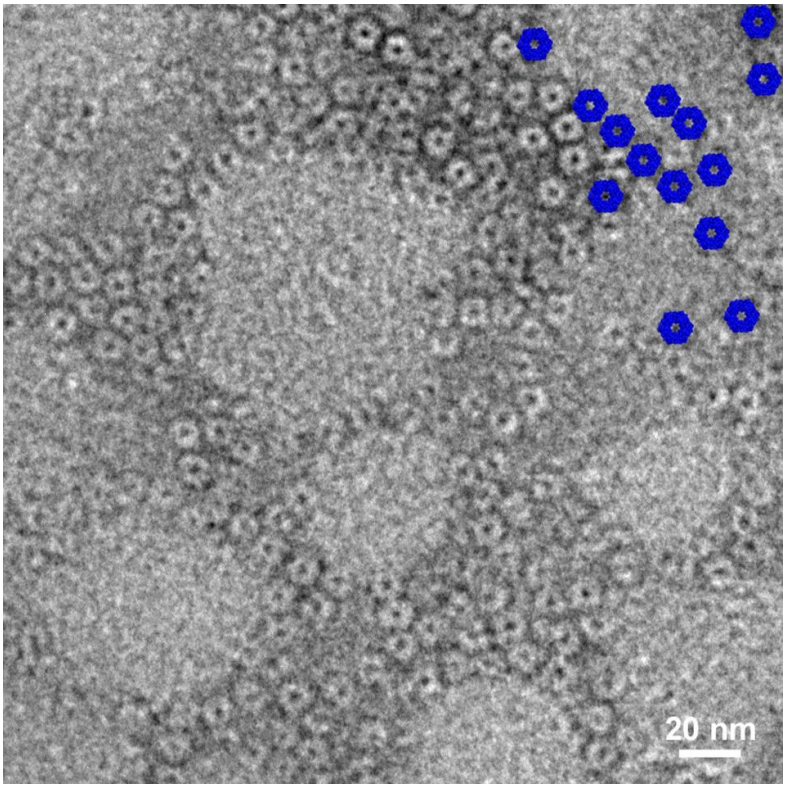
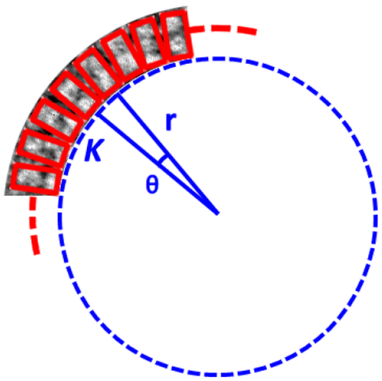


Figure S10: TEM images (negative staining) of free SP1 proteins after UV irradiation for 10 min.



$r = 0.5 \times 50 = 25$ nm

$K = r^{-1} = 25^{-1} = 0.040$ nm^{-1}

$N = 360 \div \theta = 360 \div 10.5 = 34$

Figure S11: Composition analysis of the supramolecular protein rings.

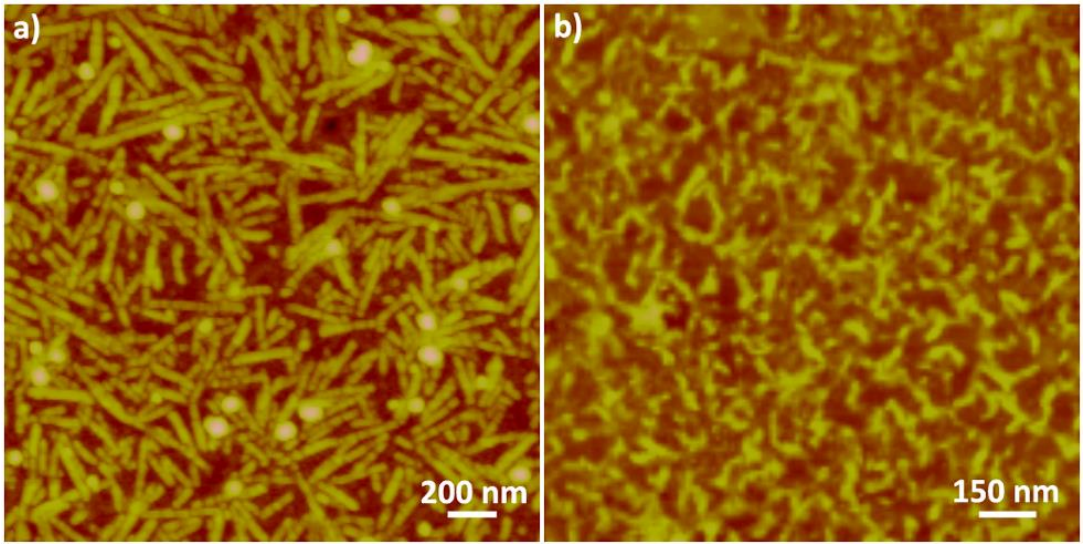


Figure S12: AFM topographic images of photocontrolled reversibly interconverted AzoPD4/SP1 nanowires. (a) AFM image of cis-AzoPD4/SP1 nanowires after VIS light irradiation. (b) AFM image of trans-AzoPD4/SP1 nanowires in image (a) after being irradiated under UV light.

Reference

[1] Sun, H.C.; Miao, L.; Li, J.X.; Fu, S.; An, G.; Si, C.Y.; Dong, Z.Y.; Luo, Q.; Yu, S.J.; Xu, J.Y.; *et al*. Self-Assembly of Cricoid Proteins Induced by “Soft Nanoparticles”: An Approach to Design Multienzyme-Cooperative Antioxidative Systems. *ACS Nano* **2015**, *9*, 5461–5469.

[2] Yu, H.J.; Nie, Y.; Dohmen, C.; Li, Y.Q.; Wagner, E. Epidermal Growth Factor PEG Functionalized PAMAM-Pentaethylenehexamine Dendron for Targeted Gene Delivery Produced by Click Chemistry. *Biomacromolecules* **2011**, *12*, 2039–2047.