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Electronic Supplementary Information (ESI)

Fluorescent Azobenzene-confined Coiled-coil Mesofibers

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Experimental

Materials

M15MA[1] *Escherichia coli* cells were a gift from David Tirrell (California Institute of Technology). Ampicillin, kanamycin, tryptic soy agar, bacto-tryptone, sodium chloride, yeast extract, ammonium chloride (NH_4Cl), dextrose monohydrate (D-glucose), magnesium sulfate ($MgSO_4$), calcium chloride ($CaCl_2$), sodium phosphate monobasic monohydrate ($NaH_2PO_4.H_2O$), sodium phosphate dibasic anhydrous (Na_2HPO_4), potassium phosphate monobasic (KH_2PO_4), sodium hydroxide (NaOH), manganese chloride tetrahydrate ($MnCl_2{\cdot}4H_2O$), cobaltous chloride hexahydrate ($CoCl_2{\cdot}6H_2O$), isopropyl b-D-1-thiogalactopyranoside (IPTG), tris-hydrochloride (Tris-HCl), sodium dodecyl sulfate (SDS), pierce bicinchoninic acid (BCA) assay kit, pierce snakeskin dialysis tubing 3.5 kDa molecular weight cut off (MWCO), and 3.5 kDa MWCO Slide-A-Lyzer MINI Dialysis Devices, black 96-well plates were purchased from Thermo Fisher Scientific. Dimethyl sulfoxide (DMSO) was purchased from Sigma Aldrich. Imidazole was purchased from Acros Organics. HiTrap immobilized metal affinity chromatography (IMAC) fast flow (FF) 5 mL column for protein purification and Whatman filter paper for transmission electron microscopy (TEM) sample preparation were purchased from GE Healthcare Life Sciences. Formvar/carbon-coated copper grids (FCF400-Cu) and 1% uranyl acetate for TEM were purchased from Electron Microscopy Sciences. Macrosep and Microsep Advance centrifugal devices 3 kDa MWCO and 0.2 mm syringe filters were purchased from Pall Corporation. Acrylamide/bis-acrylamide solution (30%) 29:1 and natural polypeptide sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) standard were purchased from Bio-Rad.

Protein Expression

The pqE30/Q plasmid was transformed into the *Escherichia Coli* strain M15MA cells, and the colonies were allowed to grow for 16 hours at 37°C on tryptic soy agar plates with kanamycin (35 μg/mL) and ampicillin (200 μg/mL) as selection antibiotics. Single colonies were selected from the plates and inoculated in 16mL of 1XM9 medium supplemented with 20 amino acids, kanamycin (35 μg/mL) and ampicillin (200 μg/mL) and incubated at 37°C, for 12 hours, at 350 rpm. The proteins were expressed in baffled flasks with 400 ml volume of the above-mentioned supplements. The cells were allowed to grow at 37°C and 350 rpm to optical density 600 nm (OD600) of ~0.8, at which the cultures were induced with IPTG (200 μg/mL) and incubated for 3 hours at same conditions. After overexpression, cells were harvested by centrifugation at 4000 rpm in an Avanti J-25 centrifuge (Beckman Coulter) for 20 minutes at 4°C and stored at -80°C until purification.

Purification

The cell pellets were thawed at 4°C and purified under denaturing conditions by resuspending the pellets in lysis buffer (6 M urea, 50 mM Tris-HCl, 500 mM NaCl, pH 8.0). The cells were lysed with ultrasonic probe sonicator (Q500 sonicator) at 45% amplitude, a pulse of 5s on and 5s off for 2 min. Cell lysate was subjected to centrifugation at 11000 x g for 50 minutes at 4°C and the supernatant was purified by syringe pump using 5mL HiTrap IMAC FF charged with 100mM $CoCl_2$. A gradient of 10-500 mM concentration of imidazole was used to elute the proteins. The pure fractions determined using a 12% SDS-PAGE were collected and subjected to stepwise dialysis using 3 M, 1.5 M and 0.75 M of urea in 50mM phosphate buffer (PB) pH 4.0 and six 5L buckets of 50 mM PB pH 4.0. The protein was concentrated to 5 mL using 3 kDa MWCO Macrosep centrifugal devices (Pall Corporation). The concentration of purified protein was determined using a BCA assay.

Synthesis of AzoCholine

AzoCholine was synthesized as described in literature.[2]

AzoCholine incorporation

To perform AzoCholine binding to Q, 200 μM of protein in 50mM PB, pH 4.0 was incubated with 800 μM of AzoCholine (1% DMSO v/v) in 50 mM PB, pH 4.0 to have a total volume of 2 mL of reaction mixture and allowed to shake on a thermomixer at 300 rpm at room temperature for 16 hours. To remove excess unbound AzoCholine, the reaction mixture was dialyzed against 50 mM PB pH 8.0 buffer using Thermo Scientific Slide-A-Lyzer MINI dialysis devices (3.5K MWCO, 2 mL) and buffer was exchanged three times.

Confocal microscopy

Samples were imaged using a Zeiss LSM 880 confocal microscope system equipped with AIRSCAN super resolution imaging. For fluorophore verification spectral imaging was conducted. A 63×/1.4 NA oil-immersion objective was used for all the images. Lab-Tek II chambered #1.5 German coverglass system was used as the imaging slide. The AzoCholine

was excited using the 405 laser, and images were taken with the detection window set between 425 and 500 nm. The 32 pinhole AIRYSCAN aperture was set at an Airy value of 1.4 in each pinhole. The 3D reconstructions were constructed using ImageJ64 1.49 in concert with Amira 5.43, employing the Volren 3D rendering routine.

Computational modelling

Small molecule docking simulations were performed to determine the interactions of *trans* vs *cis* conformations of AzoCholine with the Q helical fibers. AzoCholine conformer libraries were generated using the BioChemical Library BCL::Conformer Generation application.[3] Docking was done using Rosetta and the ligand transform protocol.[4] The docking protocol simultaneously samples ligand conformations and allows for flexibility in both side chain and backbone of the protein. Due to the long, narrow cavity of Q, 5 independent runs were carried out for both *cis* and *trans* sets of conformers where the starting position of the ligand was adjusted to scan the full length of the cavity. 500 models were generated from each starting conformation for a total of 5000 models.

Photoisomerization studies

To examine the effect of photoisomerization on the fluorescence of AzoCholine bound protein fibers (Q_{Azo}). 20µL of 50µM AoCholine bound protein was spotted on a glass slide and covered with glass cover slips of 60mm length, 24mm width, and 0.13mm thickness. The fluorescent fibers were imaged using Leica DMI3000 B microscope with filter set having excitation wavelength 402-418, 480-500 nm and emission at 445-465, 512-558 nm. The fibers were illuminated with three 10 minutes cycles of 370 nm UV light for *trans* to *cis* isomerization of the azobenzene moiety and switching it back from *cis* to *trans* state by using 460 nm blue light at 2 cm from the coverslip and then imaged via fluorescence microscope. Fibers were imaged after each illumination cycle to examine any change in fluorescence. The mean fluorescence intensity of the fibers was calculated by ImageJ.

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy

Secondary structure of Q and Q_{Azo} before and after three photoisomerization cycles was assessed using ATR-FTIR spectroscopy. The experiments were performed on Nicolet 6700 Fourier Transform Infrared spectrometer equipped with a diamond ATR accessory and a mercury cadmium telluride (MCT-A) detector. For both pre-and post-photoisomerization samples of Q and Q_{Azo}, 5µL of protein at a concentration of 50µM was spotted on the diamond accessory and allowed to dry for 3 minutes. The spectrum was obtained at room temperature by scanning the samples for a total of 128 scans across a range of 4000–400 cm^{-1} with a resolution of 0.5 cm^{-1}. The content of secondary structure for each sample was determined by deconvoluting the spectra in the amide I region (1600-1700 cm^{-1}) using PeakFit software (Version 4.12, Systat Software, Inc.).[5] The data processing involved determining the peaks using second derivative zero baseline correction and then deconvolution of peaks using Gaussian functions. Each peak was deconvoluted to a full-width at half maximum of less than 30 cm^{-1} and $r^2 \geq 0.99$.[6]

Transmission electron microscopy (TEM)

Q protein sample (2 µL) of at 50 µM in 50mM PB, pH 4.0 was spotted on copper grids. The grids were blotted using filter paper after 1 minute of incubation and then washed twice using 5 µL deionized water. 1% uranyl acetate (3 µL) was used to negatively stain the samples. TEM was performed using EI TalosL120C TEM equipped with Gatan 4k x 4k OneView camera. The nanofibers diameters were measured using ImageJ software.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used to analyze the morphology of Qazo fibers. Samples were mounted on the aluminum SEM specimen holders and then coated with palladium for 10 minutes using a Med20 Sputter coater (Baltec- Leica, Buffalo Grove, IL). Amray 1910 field emission scanning electron microscope was then used to analyze the samples at the operating voltage of 10kV. Digital images were acquired using SEM Image Display software (SEMTech Solutions, North Billerica, MA).

Widefield optical microscopy

Widefield optical microscopy was used to confirm the absence of microfibers upon addition of 1% DMSO to a solution of Q at final concentration ranges from 50 µM to 1500 µM. A microscope slide was first etched in nitric acid before being rinsed DI water and dried with N_2. 3 µL of Q was then deposited and covered with a 22 x 22 mm #1 microscope cover glass and observed with a Leica DFC310 FX 1.4 megapixel camera at 40x magnification.

Photoswitching experiment:

1mL of Qazo sample was centrifuged at 2000 × g for 5 minutes to separate the supernatant comprising protein nanoaggregates and pellet containing mesofibers. The pellet was resuspended in 1mL of PB buffer. Sample preparation and all experiments were performed under red light conditions in a dark room. UV-Vis spectra were recorded on a Varian Cary 60 Scan UV-Vis spectrometer equipped with a Peltier PCB-1500 Thermostat and an 18-cell holder using Brand disposable UV cuvettes (70-850 µL, 10 mm light path) by Brandtech Scientific Inc. Light at different wavelengths was provided by an Optoscan Monochromator with an Optosource (75 mW lamp) irradiation to establish that the photostationary state took place from the top through a fiber-optic cable. First, a spectrum of the non-irradiated sample was acquired (dark). Then, the sample was illuminated with three 200 seconds cycles of 370 nm UV light for trans to cis isomerization of the azobenzene moiety and switching it back from cis to trans state by using 460 nm blue light. To study the effect of photoisomerization cycles, UV-Vis spectra of the sample were acquired in dark after each isomerization cycle. Relative absorption of π- π* band at 340 nm for each isomerization cycle was plotted over time. Finally, to confirm observed turbidity is the result of formation of precipitated protein aggregates, TEM images were taken of a 50 µM

Qazo sample mixed in a glass vial and subjected to transilluminator plate with 230V input source for blue light (485 nm) and 240 V UV light (385 nm).

Drug Binding and Spectroscopic Fluorescence Experiment:

Q was bound to AzoCholine in 1 mL final volumes at increasing ratios of Q:AzoCholine. AzoCholine solubilized in DMSO was added to PB at a final concentration of 20 µM and 1% DMSO before adding Q at increasing concentrations from 10 to 50 µM. Samples were then dialyzed 3 x for 2 h in 3.5 MWCO Slide-A-Lyzer MINI Dialysis Devices (Thermo Scientific) to remove free AzoCholine that exhibited similar fluorescence quenching behavior to other Azobenzenes[7, 8]. Samples were then excited at 410 nm and read at 440 nm to 520 nm using a BioTek Synergy H1 microplate reader at RT on a black 96-well plate. Separately, DMSO was added to a final concentration of 1% at increasing concentrations of Q without AzoCholine. Samples were re-read after addition of 3 µL of 100 mM $NiCl_2$ for a final concentration of 100 µM. Since Q possessed significant fluorescence emission to bound AzoCholine, fluorescence maximum exhibited by Q in 1% DMSO was subtracted from fluorescence maximum exhibited by AzoCholine bound Q in 1% DMSO. To assess specificity of the pore for binding AzoCholine, 100 µM $NiCl_2$ was introduced to destroy the self-assembly of Q and thus inhibit small molecule binding to the pore as seen previously [9].

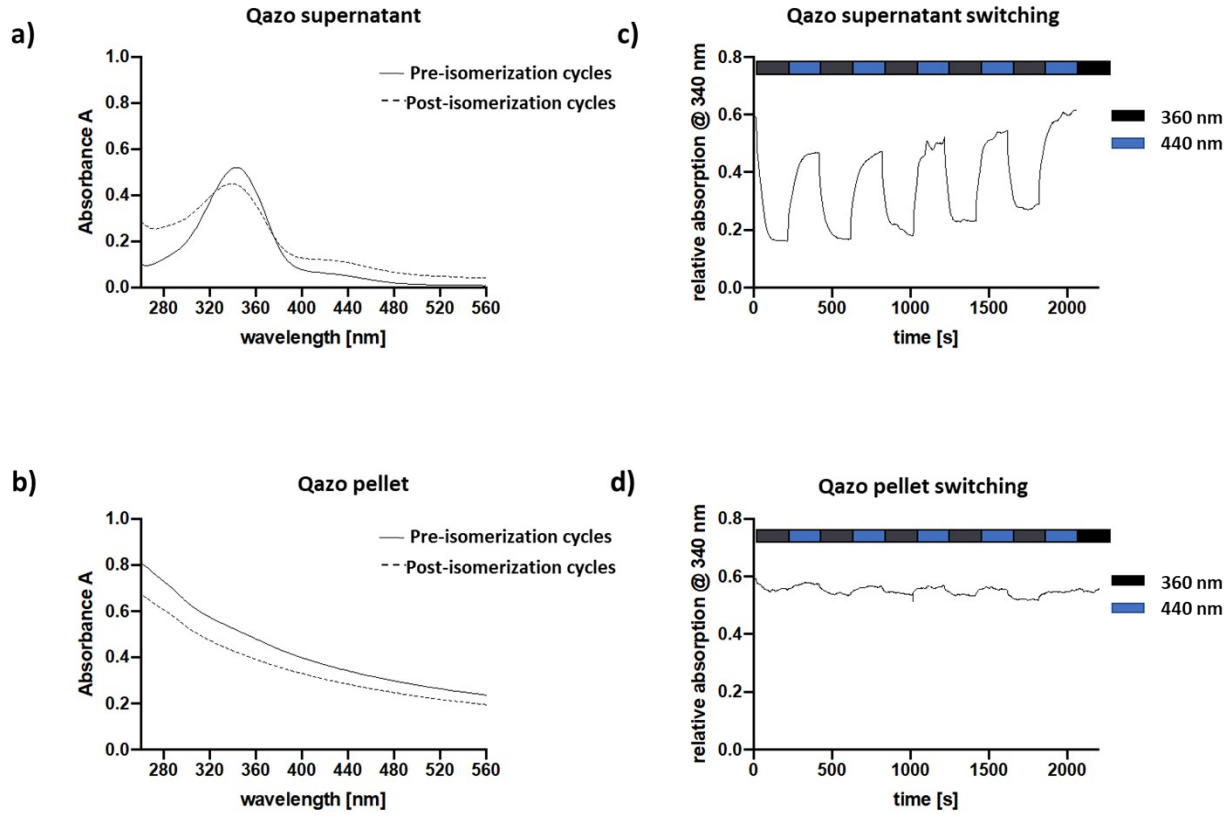

Fig. S1 Effect of photoisomerization on the absorbance spectrum of AzoCholine bound Q sample. a), b) UV/Vis absorbance measurements made prior to and post isomerization cycles of Qazo supernatant and pellet respectively. c), d) Relative absorbance of π- π* band at 340 nm measured after each 200 s of illumination with 370 nm UV and 460 nm blue light for Qazo supernatant and pellet respectively.

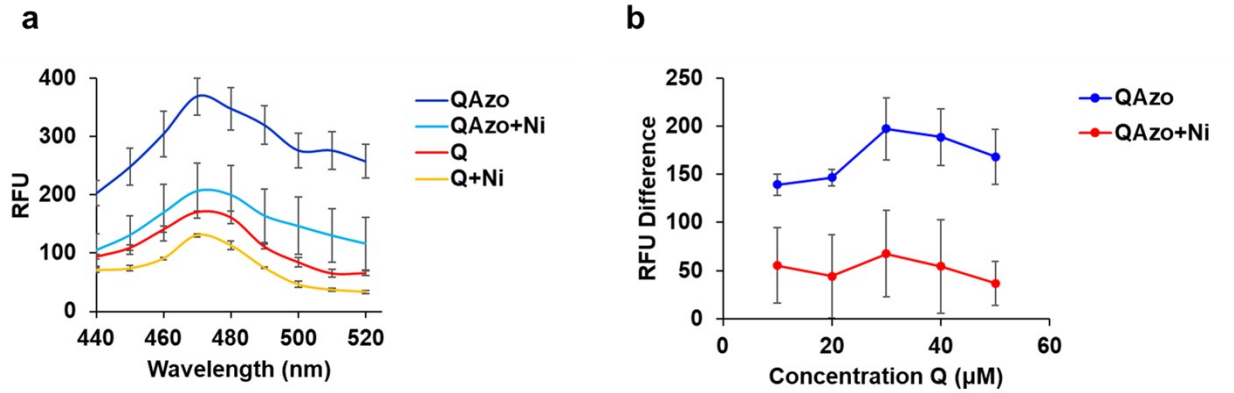

Fig. S2 a. Emission spectra from 440 nm to 520 nm of 30 µM Q and 30 µM AzoCholine bound to Q sample in 1% DMSO in the presence of 100 µM $NiCl_2$ after excitation at 410 nm. **b.** Spectroscopic fluorescence difference of AzoCholine bound Q with and without 100 µM $NiCl_2$ at increasing ratios of Q:AzoCholine. Average and standard error are the result of three trials of AzoCholine binding studies to Q and DMSO addition to Q followed by re-reading after addition of 100 µM $NiCl_2$.

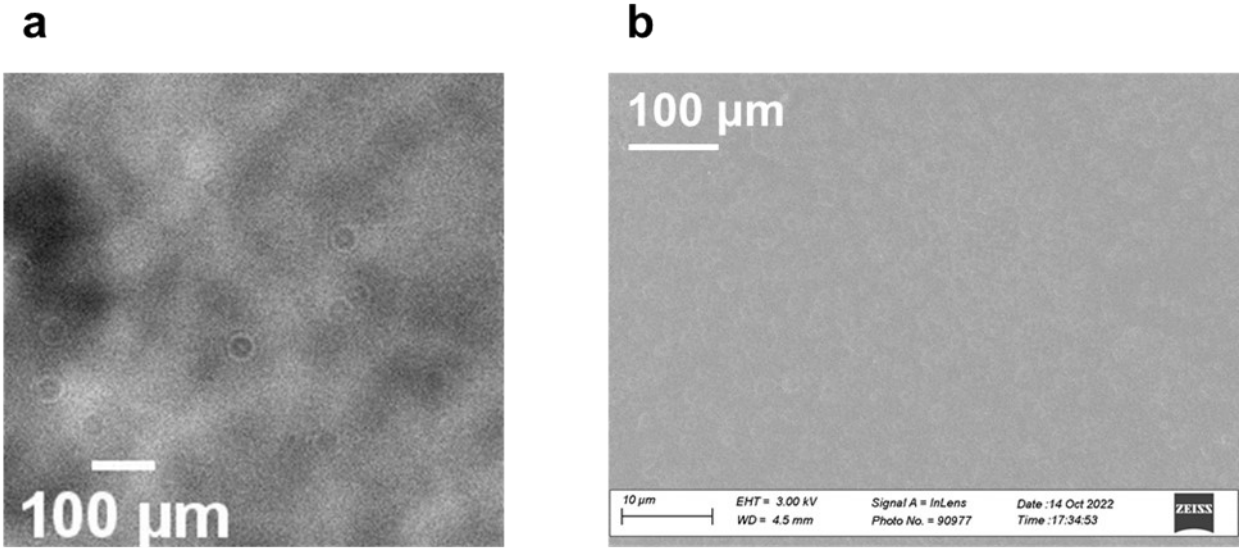

Fig. S3 Representative image of Q samples in 1% DMSO from **a.** widefield microscopy of Q samples in 1% DMSO **b.** scanning electron microscopy

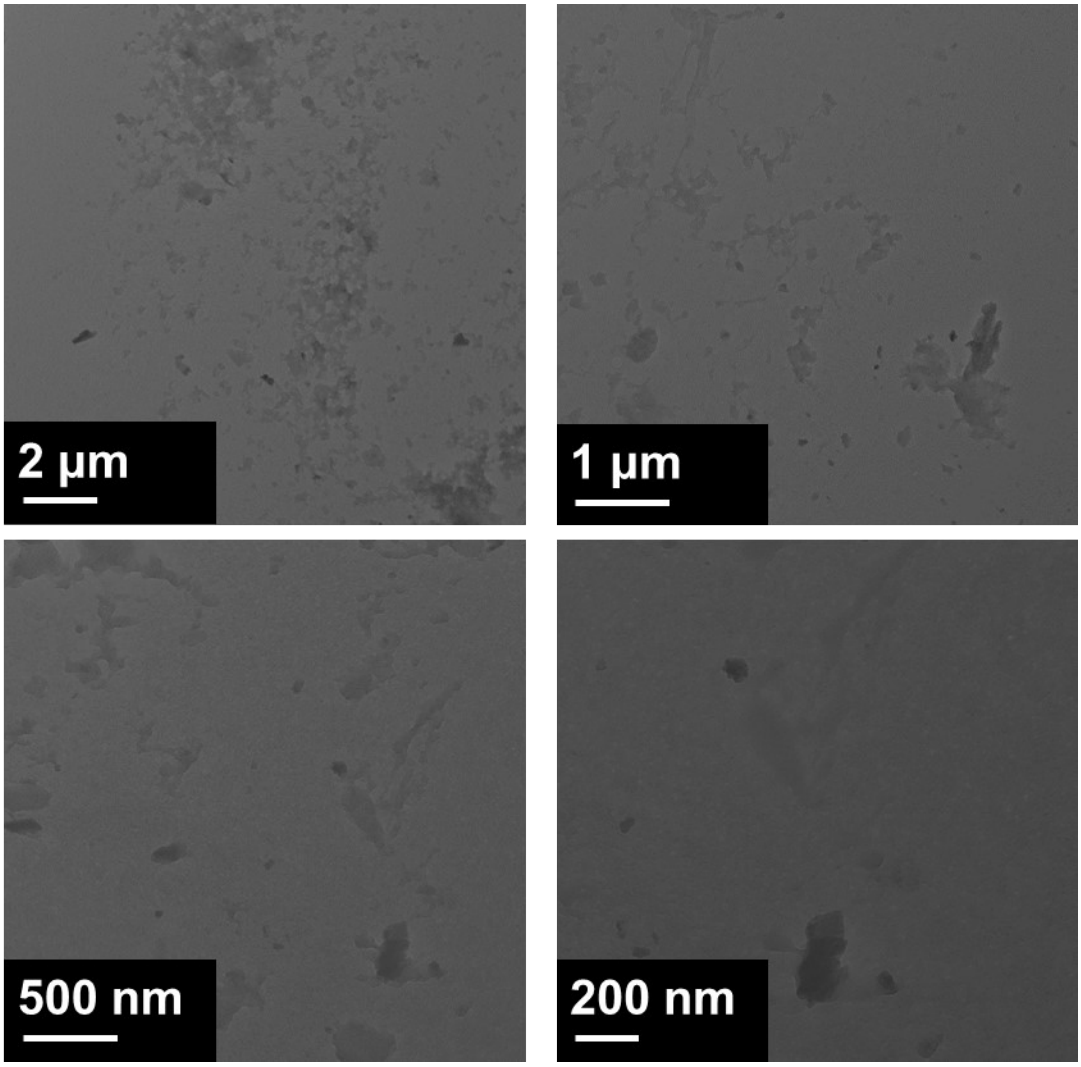

Fig. S4 Representative TEM images at various resolutions of QAzo samples in 1% DMSO with addition of 100 µM $NiCl_2$.

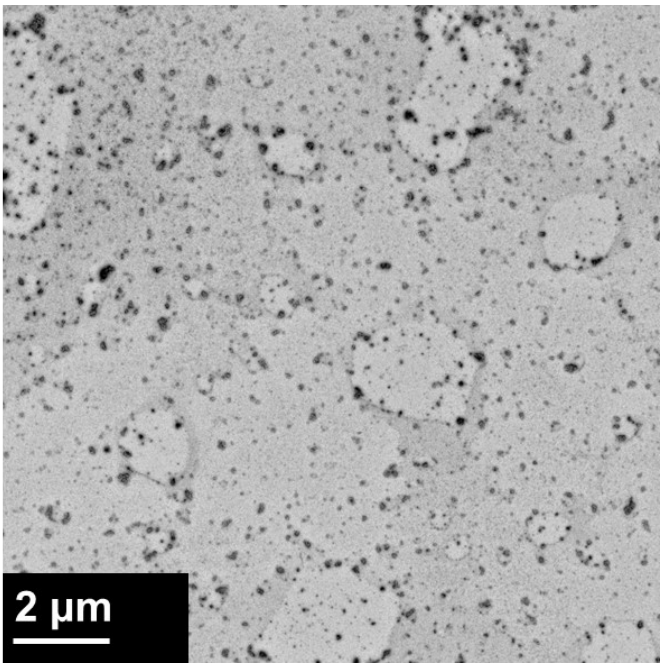

Fig. S5 Representative TEM image of Qazo after 3 cycles of photoisomerization

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