Electronic Supporting Information for

Full-colour Jabuticaba-like nanostructures via multiplex and orthogonal self-assembly of protein conjugated quantum dots with engineered biofilms

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EXPERIMENTAL SECTION

Chemicals. Cadmium nitrate tetrahydrate ($Cd(NO_3)_2 \bullet 4H_2O$, 99.8%), Cadmium oxide (CdO, 99.99+%, powder), Zinc nitrate tetrahydrate($Zn(NO_3)_2 \cdot 4H_2O$, 99.8%), Tellurium (Te, powder, -200 mesh, ≥99%, powder), Selenium (Se, powder, <100 mesh, 99.99%), Sulfur (S, 99.998% powder), Paraffin liquid (C_nH_{2n+2}, n = 16−22), Oleic acid (OLA, $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$, 90%), Sodium borohydride ($NaBH_4$, powder, ≥99%), 3-Mercaptopropionic acid (MPA, $HSCH_2CH_2CO_2H$, ≥99%), β-mercaptoethanol ($HSCH_2CH_2OH$, ≥99.0%), Isopropyl alcohol (IPA, 99%), Hexane (≥95%), Methanol (≥99.5%), Uranyl acetate dehydrate ($UO_2(OCOCH_3)_2 \bullet 2H_2O$, ≥98%), Rhodamine 110 (QY = 92% in ethanol), Rhodamine 101 (QY = 100% in ethanol + 0.01 HCl), and Coumarin 102 (QY = 76.4% in ethanol) were purchased from Sigma-Aldrich and used without further purification. Ampicillin sodium salt (Amp, $C_{16}H_{18}N_3O_4SNa$), Spectinomycin dihydrochloride (Spec, $C_{14}H_{24}N_2O_7 \cdot 2HCl \cdot 5H_2O$), Kanamycin sulfate (Kan, $C_{18}H_{36}N_4O_{11} \cdot H_2SO_4$), Chloramphenicol (Cm, $C_{11}H_{12}Cl_2N_2O_5$), and Carbenicillin disodium salt (Carb, $C_{17}H_{16}N_2Na_2O_6S$) were purchased from Fisher Scientific and used without further purification.

Genetic engineering of tag-displaying amyloid fibrils. We engineered *E. coli* bacteria to synthesize curli amyloid fibrils that were engineered to display heterologous peptides [1, 2]. We appended DNA encoding the desired peptides to the 3' end of the gene encoding the major curlin subunit, CsgA, which forms curli amyloid fibrils, and used a tightly regulated anhydrotetracycline (aTc)-inducible system [3] to express the modified *csgA* genes. DNA containing the $csgA_{SpyTag}$, $csgA_{IsopeptagC}$, $csgA_{IsopeptagN}$ and $csgA_{HisTag}$ genes (Table S1) with KpnI and MluI sticky ends were generated by PCR and KpnI/MluI digestion. These fragments were ligated with the pZA-CmR-rr12-pL(tetO)- vector[2] to create pZA-CmR-rr12-pL(tetO)-$csgA_{SpyTag}$, pZA-CmR-rr12-pL(tetO)-$csgA_{IsopeptagC}$, pZA-CmR-rr12-pL(tetO)-$csgA_{IsopeptagN}$, and pZA-CmR-rr12-pL(tetO)-$csgA_{HisTag}$ plasmids, respectively (Table S2). These plasmids were transformed into MG1655 *PRO ΔcsgA ompR234* cells to create $aTc_{Receiver}/CsgA_{SpyTag}$, $aTc_{Receiver}/CsgA_{IsopeptagC}$, $aTc_{Receiver}/CsgA_{IsopeptagN}$ and $aTc_{Receiver}/CsgA_{HisTag}$ cells, respectively (Table S3). The *PRO* cassette allows for high-level expression of the TetR protein [4], which is necessary for tight regulation of *csgA* with aTc via the pL(tetO) promoter. The endogenous *csgA* gene was knocked out (*ΔcsgA*) to ensure that all fibrils formed were composed of genetically engineered CsgA subunits. The *ompR234* mutation enabled fibril production in liquid media [5].

For cell-based synthesis of amyloid fibrils, cells were inoculated from frozen stocks into LB with chloramphenicol (30 μg/mL) and grown at 37°C with shaking for 12 hours. The cells were then spun down and the supernatant removed. The cells were re-suspended in PBS buffer. Next, cells were inoculated into 1 mL M63 glucose with aTc (100-250 ng/mL) and chloramphenicol (30 μg/mL) in 24-

well polystyrene plate wells, which had a diameter of 1.56 cm. A round glass coverslip (from Thermanox) with diameter of 1.3 cm was placed at bottom of each well. These cells were grown at 30°C with no shaking for 16-24 hours. For production of SpyTag-displaying amyloid fibrils, $aTc_{Receiver}/CsgA_{SpyTag}$ cells were inoculated at a seeding concentration of $5x10^7$ cells/mL. For production of IsopeptagC-displaying or IsopegTagN-displaying amyloid fibrils, $aTc_{Receiver}/CsgA_{IsopeptagC}$ or $aTc_{Receiver}/CsgA_{IsopeptagN}$ cells were inoculated at a seeding concentration of $5x10^7$ cells/mL. For production of fibrils displaying both SpyTag and HisTag, $aTc_{Receiver}/CsgA_{SpyTag}$ and $aTc_{Receiver}/CsgA_{HisTag}$ cells were inoculated at a concentration of $2.5x10^7$ cells/mL each and co-cultured. For production of fibrils displaying both IsopeptagC and HisTag, $aTc_{Receiver}/CsgA_{IsopeptagC}$and $aTc_{Receiver}/CsgA_{HisTag}$ cells were inoculated at a concentration of $2.5x10^7$ cells/mL each and co-cultured. For production of fibrils displaying SpyTag, IsopeptagC and IsopeptagN, $aTc_{Receiver}/CsgA_{SpyTag}$, $aTc_{Receiver}/CsgA_{IsopeptagC}$ and $aTc_{Receiver}/CsgA_{IsopeptagN}$ were inoculated at a concentration of $1.7x10^7$ cells/mL each and co-cultured (Table S2). The resulting cellular populations were washed, dislodged, and resuspended in 1XPBS buffer to obtain cells and amyloid fibrils for the organization of QDs.

Production of Cys_2-SpyCatcher, Cys_2-PilinC and PilinN-Cys_2 proteins. To produce the Cys_2-SpyCatcher protein, we introduced codons encoding two cysteine residues to the gene encoding *SpyCatcher*, expressed the protein in *E. coli*, and purified the recombinant protein [1]. Specifically, we used the QuikChange Lightning Kit (Agilent) on the pDEST14-T7-*SpyCatcher* plasmid [6] to add codons encoding two cysteine residues after the start codon of the *SpyCatcher* gene, creating the pDEST14-T7-*Cys_2-SpyCatcher* expression plasmid (Table S1). This expression plasmid was transformed into *E. coli* BL21(DE3) pLysS to create BL21(DE3) pLysS / pDEST14-T7-*Cys_2-SpyCatcher* (Table S2). For expression of Cys_2-SpyCatcher, *E. coli* strain BL21(DE3) pLysS / pDEST14-T7-*Cys_2-SpyCatcher* from a frozen stock was grown overnight in 50 mL LB-Miller with 50 μg/mL carbenicillin. Then, 10 mL of stationary phase cells were added to 1-liter LB-Miller with 50 μg/mL carbenicillin, and further grown to OD600 of 0.5-0.7 at 37°C with shaking for 3-4 hours. Finally, IPTG (0.4 mM) was added and the culture was grown with shaking for 4 hours at 30°C. The cells were collected by centrifugation and lysed. Proteins were purified with Ni-NTA Resin (Qiagen) using standard protocols. 2 mM β-mercaptoethanol was used to break disulfide bonds. Through buffer exchange with Amicon columns, the resulting Cys_2-SpyCatcher proteins were re-dispersed in 1XPBS buffer to OD280 of 0.5.

To produce the Cys_2-PilinC protein, we used the QuikChange Lightning Kit (Agilent) on the pET28a-T7-*PilinC* plasmid [7] to add codons encoding two cysteine residues after the start codon of the *PilinC* gene, creating the pET28a-T7-*Cys_2-PilinC* expression plasmid (Table S1). This expression plasmid was

transformed into *E. coli* BL21(DE3) pLysS to create BL21(DE3) pLysS / pET28a-T7-*Cys_2-PilinC* (Table S2). For expression of Cys_2-PilinC, *E. coli* strain BL21(DE3) pLysS / pET28a-T7-*Cys_2-PilinC* from a frozen stock was grown overnight in 50 mL LB-Miller with 30 μg/ml kanamycin. Then, 10 mL of stationary phase cells were added to 1-liter LB-Miller with 30 μg/ml kanamycin, and further grown to OD600 of 0.5-0.7 at 37°C with shaking for 3-4 hours. Finally, IPTG (0.4 mM) was added and the culture was grown with shaking for 4 hours at 30°C. The cells were collected by centrifugation and lysed. Proteins were purified with Ni-NTA Resin (Qiagen) using standard protocols. 2 mM β-mercaptoethanol was used to break disulfide bonds. Through buffer exchange with Amicon columns, the resulting Cys_2-PilinC proteins were re-dispersed in 1XPBS buffer to OD280 of 0.5.

To produce PilinN-Cys_2 proteins, we synthesized the protein-encoding gene (Integrated DNA Technologies) and cloned it into the pET28a vector via Gibson assembly to create pET28a-T7-*PilinN-Cys_2* (Table S1). This expression plasmid was transformed into *E. coli* BL21(DE3) pLysS to create BL21(DE3) pLysS / pET28a-T7-*PilinN-Cys_2* (Table S2). For expression of PilinN-Cys_2, *E. coli* strain BL21(DE3) pLysS / pET28a-T7-*PilinN-Cys_2* from a frozen stock was grown overnight in 50 mL LB-Miller with 30 μg/ml kanamycin. Then, 10 mL of stationary phase cells were added to 1-liter LB-Miller with 30 μg/ml kanamycin, and further grown to OD600 of 0.5-0.7 at 37°C with shaking for 3-4 hours. Finally, IPTG (0.4 mM) was added and the culture was grown with shaking for 4 hours at 30°C. The cells were collected by centrifugation and lysed. Proteins were purified with Ni-NTA Resin (Qiagen) using standard protocols. 2 mM β-mercaptoethanol was used to break disulfide bonds. Through buffer exchange with Amicon columns, the resulting PilinN-Cys_2 proteins were re-dispersed in 1XPBS buffer to OD280 of 0.5.

For further purification of proteins, the eluted Cys_2-SpyCatcher, Cys_2-PilinC, and PilinN-Cys_2 in elution buffer (NPI-500, 50 mM NaH_2PO_4, 300 mM NaCl, 500 mM imidazole, pH 8.0) were loaded into 0.5 mL Amicon filters (MWCO 3 KDa), 1XPBS buffer was added to the filters to make the total volumes 500 μL, and the samples were subjected to centrifugation at 11,000 rpm for 10 minutes. The washing (each wash process was performed with 400 μL of 1XPBS buffer) and centrifugation steps were repeated three times. This ultrafiltration process removed imidazole from the protein solutions. The optimized yield of the recombinant proteins is around 10 mg/L. Samples were electrophoresed on a 12% SDS polyacrylamide gel and stained with Coomassie Blue following standard staining protocols. After destaining with destaining solutions (a mixed solution with v/v of H_2O 50%, methanol 40%, acetate acid 10%), the gels were imaged using a Bio-Rad ChemiDoc MP system.

CdSe/CdS core/shell QDs conjugated with proteins. We used protocols published previously for the

synthesis of oleic-acid capped CdSe QDs, and then performed ligand exchange to produce water-soluble CdSe QDs [8]. For a typical conjugation experiment, a 20 µL water-soluble CdSe QD solution (OD_{625} of 0.01) was added to 80 µL of DI-water. Then, 10 µL of Cd^{2+} stock solution (25 mM) and 20 µL of MPA stock solution (25 mM) were added, vortexed, and gently sonicated in a 1.5 mL plastic tube. The pH was adjusted to 12.2 with 1M NaOH. Next, 20 µL of purified Cys_2-SpyCatcher stock solution (OD_{280} of ~0.5) was added and gently vortexed. The mixture was heated in an Eppendorf thermomixer at 90°C and 600 rpm for 30 minutes, and then cooled by submerging in an ice-water bath. To remove unconjugated Cys_2-SpyCatcher from the QD-SpyCatcher conjugation, the reaction mixture was loaded into a 0.5 mL Amicon filter (MWCO 30 KDa) and 1XPBS buffer was added to the filter to make the total volume of the solution equal to 500 µL. The sample was subjected to centrifugation at 7,000 rpm for 7 minutes. The washing (each washing was performed by adding 400 µL of 1XPBS buffer) and centrifugation steps were repeated three times. For CdSe/CdS core/shell QDs conjugated with Cys_2-PilinC, 20 µL of purified Cys_2-PilinC stock solution (OD_{280} of ~0.5) was added to water-soluble CdSe QD precursor solution and processed as described above.

CdTe/CdS core/shell QDs conjugated with proteins. The synthesis of the CdTe/CdS core/shell QDs followed a previously reported protocol [9]. A series of 1 mL CdTe QD precursor solutions were loaded into 1.5 mL plastic tubes, which were placed in an Eppendorf thermomixer at 90°C and 600 rpm for various reactions times, and then cooled by submerging the tubes in an ice-water bath. To produce green-emitting CdTe/CdS QDs (with a photoluminescence emission peak at 510 nm), the heating time was 20 minutes. To produce red CdTe/CdS QDs (with a photoluminescence emission peak at 630 nm), the heating time was 50 minutes. Then, 50 µL of green or red QD solutions was added to 50 µL of DI-water in clean plastic tubes, respectively. Then, 20 µL of purified Cys_2-SpyCatcher stock solution (OD_{280} of ~0.5) was also added to each tube and gently vortexed. The mixture was heated in an Eppendorf thermomixer at 90°C and 600 rpm for another 30 minutes, and then cooled down by submerging it in an ice-water bath. The solutions were then loaded into 0.5 mL Amicon filters (MWCO 30 KDa or 100 KDa), 400 µL 1XPBS buffer was added to the filters, and the samples were subjected to centrifugation at 7,000 rpm for 7 minutes. The washing (each washing was performed with 400 µL 1XPBS buffer) and centrifugation steps were repeated three times. This ultrafiltration process removed unconjugated Cys_2-SpyCatcher from the resulting green CdTe/CdS QD-SpyCatcher conjugates with an emission peak at 530 nm and red CdTe/CdS QD-SpyCatcher conjugates with an emission peak at 650 nm. The final samples were highly fluorescent and stable in 1XPBS buffer. For CdTe/CdS core/shell QDs conjugated with Cys_2-PilinC, 20 µL of purified Cys_2-PilinC stock solution (OD_{280} of ~0.5) was used and processed similarly as the Cys_2-SpyCatcher.

ZnCdSe/ZnS core/shell QDs conjugated with proteins. The synthesis of core ZnCdSe QDs with blue emission at 440 nm followed a previously reported protocol [10]. Then, 1000 µL of core solutions in a plastic tube were mixed with ZnS precursors (100 µL 25 mM $Zn(NO_3)_2$, 100 µL 25 mM MPA), and the pH was tuned to 12.2 using 1 M NaOH. Then, 400 µL of purified PilinN-Cys_2 stock solution (OD_{280} of ~0.5) was added to each tube and gently vortexed. The mixture was heated in an Eppendorf thermomixer at 90°C and 600 rpm for another 30 minutes, and then cooled down by submerging it in an ice-water bath. The solutions were then loaded into 0.5 mL Amicon filters (MWCO 100 KDa), and subjected to centrifugation at 7,000 rpm for 7 minutes. The washing (each was performed with 400 µL 1XPBS buffer) and centrifugation steps were repeated three times. This ultrafiltration process removed unconjugated PilinN-Cys_2 from the ZnCdSe/ZnS core/shell QDs-PilinN-Cys_2 conjugates. The final samples were highly fluorescent and stable in 1XPBS buffer.

Self-assembly of QD-protein conjugates with tag-displaying amyloid fibrils. For self-assembly of QD-SpyCatcher conjugates with SpyTag-displaying amyloid fibrils, approximately 2 µL of conjugates were mixed with 100 µL of tag-displaying amyloid fibrils in 1XPBS in 600 µL plastic tubes, followed by incubation at room temperature for 30 minutes. A similar protocol was followed for the self-assembly of the QD-PilinC conjugates with $CsgA_{IsopeptagC}$ amyloid fibrils. Then, 10 µL of the mixed solution was loaded onto TEM grids (Formvar/Carbon 200 mesh Nickel) for 30 seconds. The grids were subsequently washed twice with 10 µL droplets of 1XPBS buffer and DI H_2O, followed by negative staining with uranyl acetate, and then by drying in air before TEM imaging. The TEM samples were prepared and imaged as before.

Characterization details. Ultraviolet-Visible (UV-Vis) absorption spectra were recorded at room temperature with a Varian Cary 6000i spectrophotometer. Photoluminescence spectra were measured at room temperature using a NanoLog spectrometer manufactured by HORIBA Jobin Yvon. We used a cross-calibrated method [9,10] to measure the photoluminescence quantum yield of QD-protein conjugates. The standard dyes used in the experiments were Rhodamine 101 (QY = 92% in ethanol), Rhodamine 110 (QY = 100% in ethanol + 0.01 HCl), Coumarin 102 (QY=76.4% in ethanol). Standard 10 mm path length quartz fluorescence cuvettes were used for all measurements. Fluorescence spectra of QD-protein conjugates and dye were taken under identical spectrometer conditions. The optical density was kept below 0.1 at the excitation wavelength, and the slope of the line generated by plotting the integrated fluorescence intensities against the absorption for multiple concentrations of the QD-protein conjugates and dyes were used to calculate the quantum yields.[(9)]{Deng, 2010 #30}{Deng, 2012 #5} For TEM imaging of the bacteria-QD samples, 10 µL of sample solution was

placed on a TEM grid (Electron Microscopy Sciences, #FCF200-Ni50) and maintained for 30 seconds. The solution was then wiped away with a filter paper, and washed with two drops (10 µL) of 1XPBS buffer. The resulting grids were negative stained with 2% uranyl acetate solution for 30 seconds and air-dried. For TEM imaging of the QDs before organization via amyloid fibrils, 10 µL of sample solution was placed on an ultra-thin carbon TEM grid (Ted Pella, #01822-F) and dried in air. TEM imaging and energy dispersive X-ray spectroscopy (EDS) were performed on a JEOL JEM 2010F electron microscope operating at 200 kV.

Table S1. Synthetic genes used in this work.

Part Name	Part Type	Sequence	Source
csgA	Gene for the wild-type CsgA amyloid material subunit	atg aaa ctt tta aaa gta gca gca att gca gca atc gta ttc tcc ggt agc gct ctg gca ggt gtt gtt cct cag tac ggc ggc ggc ggt aac cac ggt ggt ggc ggt aat aat agc ggc cca aat tct gag ctg aac att tac cag tac ggt ggc ggt aac tct gca ctt gct ctg caa act gat gcc cgt aac tct gac ttg act att acc cag cat ggc ggc ggt aat ggt gca gat gtt ggt cag ggc tca gat gac agc tca atc gat ctg acc caa cgt ggc ttc ggt aac agc gct act ctt gat cag tgg aac ggc aaa aat tct gaa atg acg gtt aaa cag ttc ggt ggt ggc aac ggt gct gca gtt gac cag act gca tct aac tcc tcc gtc aac gtg act cag gtt ggc ttt ggt aac aac gcg acc gct cat cag tac taa	*1*
$csgA_{HisTag}$	Gene for the CsgA amyloid material subunit with one 7XHisTag before the first repeat domain, and another 7XHisTag after the last repeat domain	atg aaa ctt tta aaa gta gca gca att gca gca atc gta ttc tcc ggt agc gct ctg gca ggt gtt gtt cct cag tac ggc ggc ggc ggt aac cac ggt ggt ggc ggt aat aat agc ggc cca aat cac cat cac cat cac cac cat tct gag ctg aac att tac cag tac ggt ggc ggt aac tct gca ctt gct ctg caa act gat gcc cgt aac tct gac ttg act att acc cag cat ggc ggc ggt aat ggt gca gat gtt ggt cag ggc tca gat gac agc tca atc gat ctg acc caa cgt ggc ttc ggt aac agc gct act ctt gat cag tgg aac ggc aaa aat tct gaa atg acg gtt aaa cag ttc ggt ggt ggc aac ggt gct gca gtt gac cag act gca tct aac tcc tcc gtc aac gtg act cag gtt ggc ttt ggt aac aac gcg acc gct cat cag tac cac cat cac cat cac cac cat taa	*2*
$csgA_{SpyTag}$	Gene for the CsgA amyloid material subunit with appended SpyTag	atg aaa ctt tta aaa gta gca gca att gca gca atc gta ttc tcc ggt agc gct ctg gca ggt gtt gtt cct cag tac ggc ggc ggc ggt aac cac ggt ggt ggc ggt aat aat agc ggc cca aat tct gag ctg aac att tac cag tac ggt ggc ggt aac tct gca ctt gct ctg caa act gat gcc cgt aac tct gac ttg act att acc cag cat ggc ggc ggt aat ggt gca gat gtt ggt cag ggc tca gat gac agc tca atc gat ctg acc caa cgt ggc ttc ggt aac agc gct act ctt gat cag tgg aac ggc aaa aat tct gaa atg acg gtt aaa cag ttc ggt ggt ggc aac ggt gct gca gtt gac cag act gca tct aac tcc tcc gtc aac gtg act cag gtt ggc ttt ggt aac aac gcg acc gct cat cag tac ggc ggg ggc tcc ggc ggg ggc tcc gcg cac atc gtt atg gtc	*2*

		gat gca tat aaa ccc acc aaa taa	
csgA$_{IsopeptagC}$	Gene for the CsgA amyloid material subunit with appended IsopeptagC	atg aaa ctt tta aaa gta gca gca att gca gca atc gta ttc tcc ggt agc gct ctg gca ggt gtt gtt cct cag tac ggc ggc ggc ggt aac cac ggt ggt ggc ggt aat aat agc ggc cca aat tct gag ctg aac att tac cag tac ggt ggc ggt aac tct gca ctt gct ctg caa act gat gcc cgt aac tct gac ttg act att acc cag cat ggc ggc ggt aat ggt gca gat gtt ggt cag ggc tca gat gac agc tca atc gat ctg acc caa cgt ggc ttc ggt aac agc gct act ctt gat cag tgg aac ggc aaa aat tct gaa atg acg gtt aaa cag ttc ggt ggt ggc aac ggt gct gca gtt gac cag act gca tct aac tcc tcc gtc aac gtg act cag gtt ggc ttt ggt aac aac gcg acc gct cat cag tac gga ggt gga agt ggc ggc gga agt acc gac aaa gat atg act atc acc ttc acg aat aaa aaa gac gcg gaa taa	This work
csgA$_{IsopeptagN}$	Gene for the CsgA amyloid material subunit with appended IsopeptagN	atg aaa ctt tta aaa gta gca gca att gca gca atc gta ttc tcc ggt agc gct ctg gca ggt gtt gtt cct cag tac ggc ggc ggc ggt aac cac ggt ggt ggc ggt aat aat agc ggc cca aat tct gag ctg aac att tac cag tac ggt ggc ggt aac tct gca ctt gct ctg caa act gat gcc cgt aac tct gac ttg act att acc cag cat ggc ggc ggt aat ggt gca gat gtt ggt cag ggc tca gat gac agc tca atc gat ctg acc caa cgt ggc ttc ggt aac agc gct act ctt gat cag tgg aac ggc aaa aat tct gaa atg acg gtt aaa cag ttc ggt ggt ggc aac ggt gct gca gtt gac cag act gca tct aac tcc tcc gtc aac gtg act cag gtt ggc ttt ggt aac aac gcg acc gct cat cag tac gga ggt gga agt ggc ggc gga agt gct aca aca gtt cac ggg gag act gtt gta aac gga gcc aaa cta aca gtt aca aaa aac ctt gat tta gtt aat agc aat gca taa	This work
Cys$_2$-SpyCatcher	Gene for the Cys$_2$-SpyCatcher protein	atg tgt tgt tcg tac tac cat cac cat cac cat cac gat tac gac atc cca acg acc gaa aac ctg tat ttt cag ggc gcc atg gtt gat acc tta tca ggt tta tca agt gag caa ggt cag tcc ggt gat atg aca att gaa gaa gat agt gct acc cat att aaa ttc tca aaa cgt gat gag gac ggc aaa gag tta gct ggt gca act atg gag ttg cgt gat tca tct ggt aaa act att agt aca tgg att tca gat gga caa gtg aaa gat ttc tac ctg tat cca gga aaa tat aca ttt gtc gaa acc gca gca cca gac ggt tat gag gta gca act gct att acc ttt aca gtt aat gag caa ggt cag gtt act gta aat ggc aaa gca act aaa ggt gac gct cat att taa	[2]

Cys_2-PilinC	Gene for the Cys_2-PilinC protein	atg tgt tgt ggc agc agc cat cat cat cat cat cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct aca aca gtt cac ggg gag act gtt gta aac gga gcc aaa cta aca gtt aca aaa aac ctt gat tta gtt aat agc aat gca tta att cca aat aca gat ttt aca ttt aaa atc gaa cct gat act act gtc aac gaa gac gga aat aag ttt aaa ggt gta gct ttg aac aca ccg atg act aaa gtc act tac acc aat tca gat aaa ggt gga tca aat acg aaa act gca gaa ttt gat ttt tca gaa gtt act ttt gaa aaa cca ggt gtt tat tat tac aaa gta act gag gag aag ata gat aaa gtt cct ggt gtt tct tat gat aca aca tct tac act gtt caa gtt cat gtc ttg tgg aat gaa gag caa caa aaa cca gta gct act tat att gtt ggt tat aaa gaa ggt agt aag gtg cca att cag ttc aaa aat agc tta gat tct act aca tta acg gtg aag aaa aaa gtt tca ggt acc ggt gga gat cgc tct aaa gat ttt aat ttt ggt ctg act tta aaa gca aat cag tat tat aag gcg tca gaa aaa gtc atg att gag aag aca act aaa ggt ggt caa gct cct gtt caa aca gag gct agt ata gat caa ctc tat cat ttt acc ttg aaa gat ggt gaa tca atc aaa gtc aca aat ctt cca gta ggt gtg gat tat gtt gtc act gaa gac gat tac aaa tca gaa aaa tat aca acc aac gtg gaa gtt agt cct caa gat gga gct gta aaa aat atc gca ggt aat tca act gaa caa gag aca tct act gat aaa gat atg acc att taa	This work

PilinN-Cys$_2$	Gene for the *PilinN-Cys$_2$* protein	atg ggc agc agc cat cat cat cat cat cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gga tta att cca aat aca gat ttt aca ttt aaa atc gaa cct gat act act gtc aac gaa gac gga aat aag ttt aaa ggt gta gct ttg aac aca ccg atg act aaa gtc act tac acc aat tca gat aaa ggt gga tca aat acg aaa act gca gaa ttt gat ttt tca gaa gtt act ttt gaa aaa cca ggt gtt tat tat tac aaa gta act gag gag aag ata gat aaa gtt cct ggt gtt tct tat gat aca aca tct tac act gtt caa gtt cat gtc ttg tgg aat gaa gag caa caa aaa cca gta gct act tat att gtt ggt tat aaa gaa ggt agt aag gtg cca att cag ttc aaa aat agc tta gat tct act aca tta acg gtg aag aaa aaa gtt tca ggt acc ggt gga gat cgc tct aaa gat ttt aat ttt ggt ctg act tta aaa gca aat cag tat tat aag gcg tca gaa aaa gtc atg att gag aag aca act aaa ggt ggt caa gct cct gtt caa aca gag gct agt ata gat caa ctc tat cat ttt acc ttg aaa gat ggt gaa tca atc aaa gtc aca aat ctt cca gta ggt gtg gat tat gtt gtc act gaa gac gat tac aaa tca gaa aaa tat aca acc aac gtg gaa gtt agt cct caa gat gga gct gta aaa aat atc gca ggt aat tca act gaa caa gag aca tct act gat aaa gat atg acc att act ttt aca aat aaa aaa gac ttt gaa gga tca gga cat cac cat cac cat cac tgt tgt taa	This work

Table S2. Plasmids used in this work.

Plasmid name	Plasmid ID	Description	Source
pZA-CmR-rr12-pL(tetO)-*csgA*$_{HisTag}$	pAYC003	p15A origin, Cm resistance, rr12 riboregulator, pL(tetO) promoter, *csgA*$_{HisTag}$ output gene	[2]
pDEST14-T7-*Cys*$_2$*-SpyCatcher*	pAYC016	pBR322 origin, Amp resistance, T7 promoter, *Cys*$_2$*-SpyCatcher* output gene	[2]
pET28a-T7-*Cys*$_2$*-PilinC*	pAYC019	pBR322 origin, Kan resistance, T7 promoter, *Cys*$_2$*-PilinC* output gene	This work
pET28a-T7- *PilinN-Cys*$_2$	pAYC020	pBR322 origin, Kan resistance, T7 promoter, *PilinN-Cys*$_2$ output gene	This work
pZA-CmR-rr12-pL(tetO)-*csgA*$_{SpyTag}$	pAYC021	p15A origin, Cm resistance, rr12 riboregulator, pL(tetO) promoter, *csgA*$_{SpyTag}$ output gene	This work
pZA-CmR-rr12-pL(tetO)-*csgA*$_{IsopeptagC}$	pAYC022	p15A origin, Cm resistance, rr12 riboregulator, pL(tetO) promoter, *csgA*$_{IsopepTagC}$ output gene	This work
pZA-CmR-rr12-pL(tetO)-*csgA*$_{IsopeptagN}$	pAYC023	p15A origin, Cm resistance, rr12 riboregulator, pL(tetO) promoter, *csgA*$_{IsopepTagN}$ output gene	This work

Table S3. Cell strains used in this work.

Strain name	Stain ID	Description	Antibiotic Resistance	Source
MG1655 *PRO ΔcsgA ompR234*	fAYC002	*E. coli* host strain with *PRO* cassette (P_{laci}^{q}/*lacI*, P_{N25}/*tetR*, $Spec^{R}$) that constitutively expresses TetR and LacI repressors, with knock-out of endogenous *csgA*, and with a *ompR234* allele that confers the ability to produce fibrils in liquid M63 minimal media.	Spec, Kan	[1]
$aTc_{Receiver}$/ $CsgA_{HisTag}$	fAYC003	*E. coli* strain that expresses $CsgA_{His}$ under tight regulation by an anhydrotetracycline (aTc) inducer-responsive riboregulator. Made by transforming pZA-CmR-rr12-pL(tetO)-$csgA_{HisTag}$ plasmid into MG1655 *PRO* Δ*csgA ompR234*.	Spec, Kan, Cm	[2]
BL21(DE3) pLysS / pDEST14-T7-Cys_2-*SpyCatcher*	fAYC016	*E. coli* strain that expresses Cys_2-SpyCatcher when induced by IPTG. Made by transforming pDEST14-T7-Cys_2-*SpyCatcher* into BL21(DE3) pLysS.	Cm, Amp	[2]
BL21(DE3) pLysS / pET28a-T7-Cys_2-*PilinC*	fAYC022	*E. coli* strain that expresses Cys_2-PilinC when induced by IPTG. Made by transforming pET28a-T7-Cys_2-*PilinC* into BL21(DE3) pLysS.	Cm, Kan	This work
BL21(DE3) pLysS / pET28a-T7-*PilinN*-Cys_2	fAYC023	*E. coli* strain that expresses PilinN-Cys_2 when induced by IPTG. Made by transforming pET28a-T7-*PilinN*-Cys_2 into BL21(DE3) pLysS.	Cm, Kan	This work
$aTc_{Receiver}$/ $CsgA_{SpyTag}$	fAYC024	*E. coli* strain that expresses $CsgA_{SpyTag}$ under tight regulation by an aTc inducer-responsive riboregulator. Made by transforming pZA-CmR-rr12-pL(tetO)-$csgA_{SpyTag}$ into	Spec, Kan, Cm	This work

		MG1655 *PRO ΔcsgA ompR234.*		
$aTc_{Receiver}$/ $CsgA_{IsopeptagC}$	fAYC025	*E. coli* strain that expresses $CsgA_{IsopeptagC}$ under tight regulation by an aTc inducer-responsive riboregulator. Made by transforming pZA-CmR-rr12-pL(tetO)-*csgA*$_{IsopeptagC}$ into MG1655 *PRO ΔcsgA ompR234.*	Spec, Kan, Cm	This work
$aTc_{Receiver}$/ $CsgA_{IsopeptagN}$	fAYC026	*E. coli* strain that expresses $CsgA_{IsopeptagN}$ under tight regulation by an aTc inducer-responsive riboregulator. Made by transforming pZA-CmR-rr12-pL(tetO)-*csgA*$_{IsopeptagN}$ into MG1655 *PRO ΔcsgA ompR234.*	Spec, Kan, Cm	This work

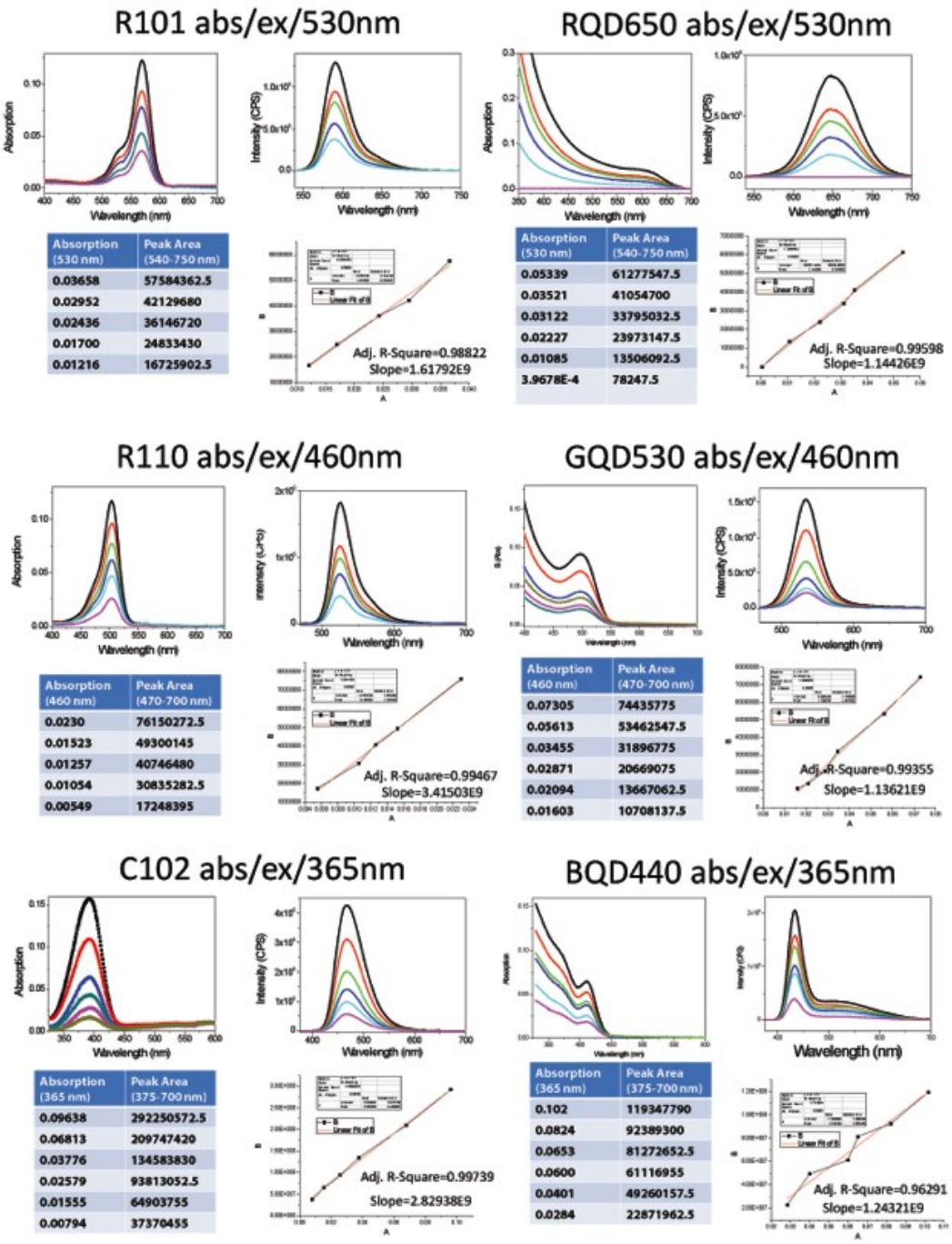

Figure S1. Determination of fluorescence quantum yields (QY) of the red CdTe/CdS-SpyCatcher (RQD650, QY = 67%), green CdTe/CdS-PilinC (GQD530, QY = 30%), and blue ZnCdSe/ZnS-PilinN (BQD440, QY = 32%) conjugates. To determine these quantum yields, we obtained UV-absorption spectra, corresponding photoluminescence spectra, and corresponding slope determinations for the fluorescent standards: Rhodamine 110 (R110, QY = 100% in ethanol + 0.01 HCl), Rhodamine 101 (R101, QY = 92% in ethanol), and Coumarin 102 (C102, QY = 76.4% in ethanol).

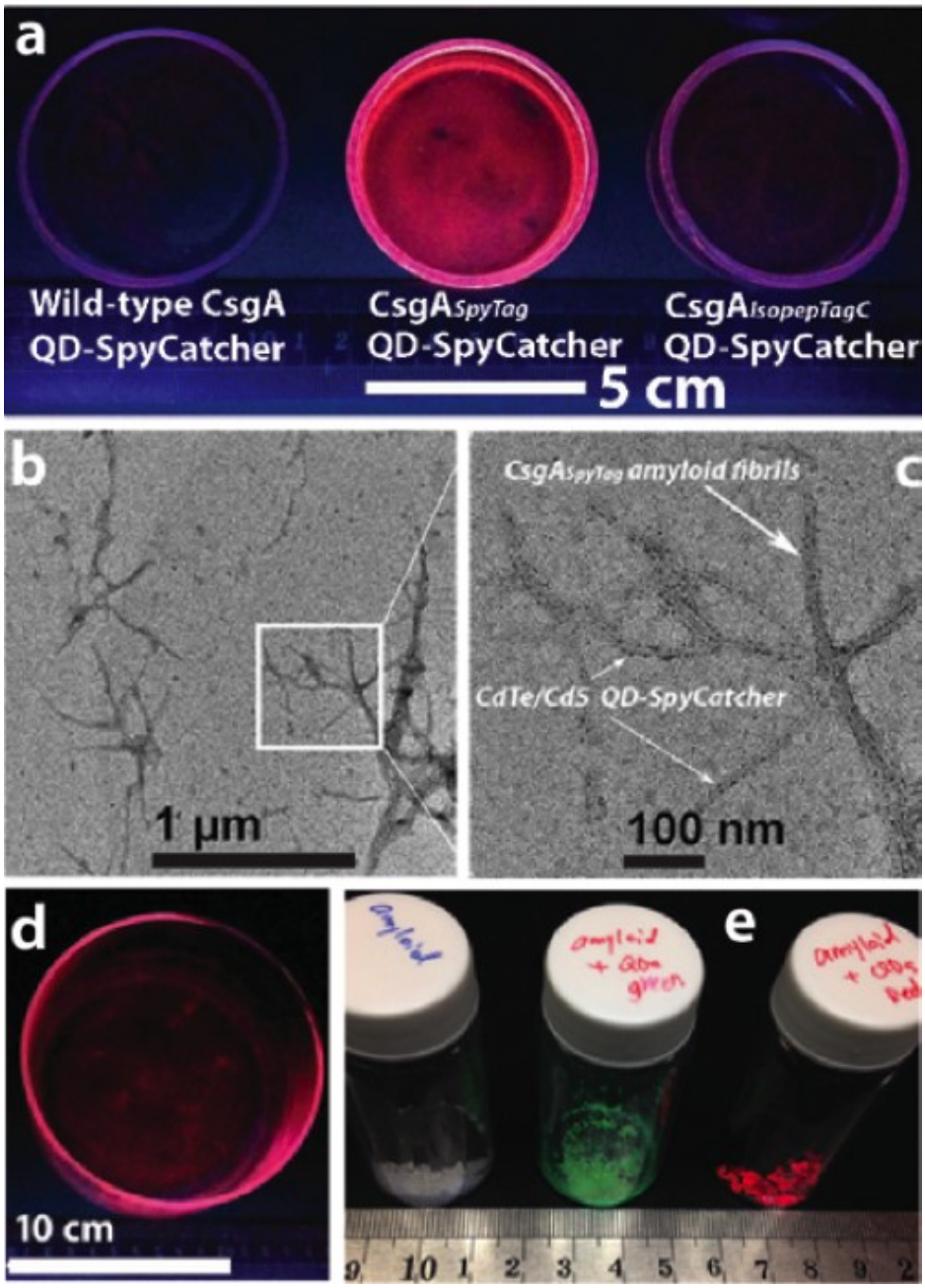

Figure S2. (**a**) Photographs of RQD-SpyCatcher conjugates assembled with *E. coli* expressing wild-type (wt) CsgA, CsgASpyTag, or CsgAIsopeptagC amyloid fibrils as well as GQD-PilinC conjugates self-assembled with CsgAIsopeptagC amyloid fibrils. (**b&c**) TEM images from the middle glass dish in image **a** demonstrate $CsgA_{SpyTag}$ amyloid fibrils organizing CdTe/CdS QD-SpyCatcher conjugates. Image **c** is from the white rectangle marked in (**b**). (**d**) Photographs (illuminated with a 365 nm UV lamp in the dark) of the red CdTe/CdS QD-SpyCatcher conjugates assembled on $CsgA_{SpyTag}$ amyloid fibrils synthesized by living cells grown in glass dishes. (**e**) Photographs of freeze-dried $CsgA_{SpyTag}$ amyloid fibrils with living cells on their own (left), as well as with green (middle) or red (right) CdTe/CdS QD-SpyCatcher conjugates under illumination with a 365 nm UV lamp under ambient light.

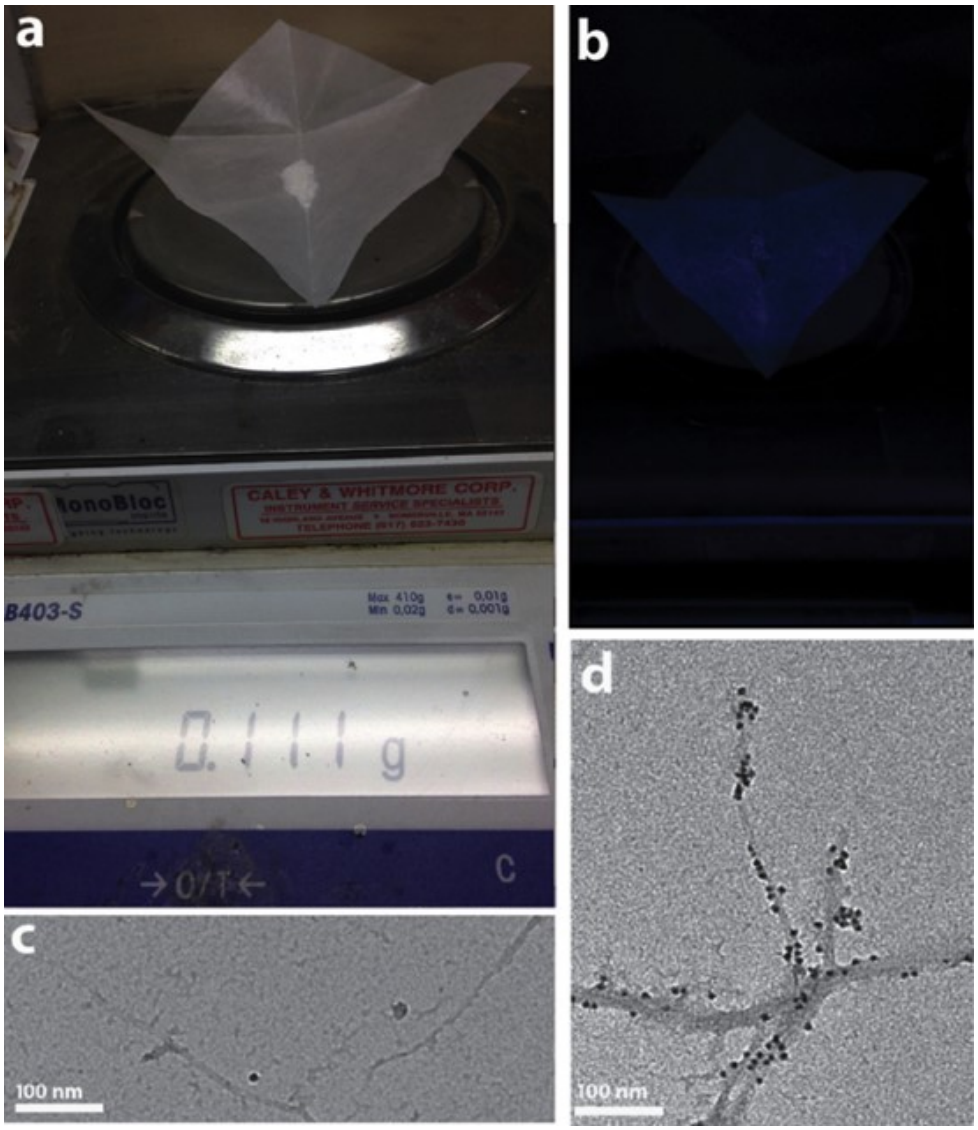

Figure S3. Large-scale synthesis of $CsgA_{SpyTag}$ amyloid fibrils by living cells. (**a&b**) Pictures of freeze-dried $CsgA_{SpyTag}$ amyloid fibril powders from five batches of one-liter-scale bacterial cultures under (**a**) ambient light and (**b**) 365 nm UV light. **c&d,** TEM images of freeze-dried $CsgA_{SpyTag}$ amyloid fibrils with living cells dispersed in 1XPBS buffer and then assembled with (**c**) unconjugated CdSe/CdS core/shell QDs and (**d**) CdSe/CdS QD-SpyCatcher conjugates.

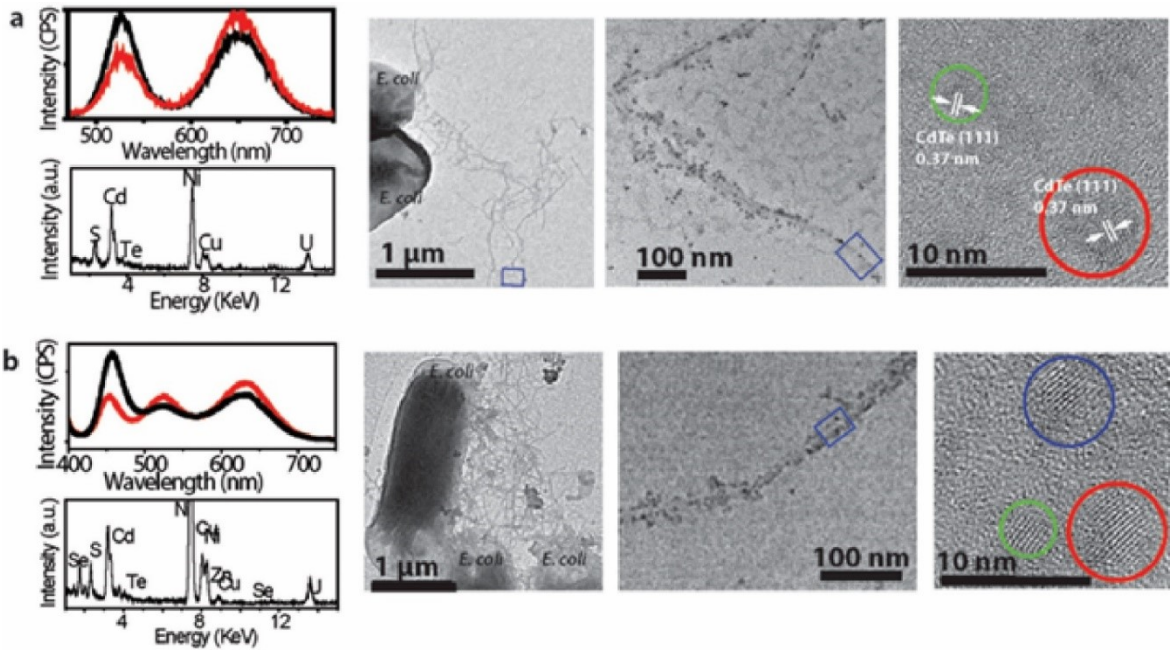

Figure S4. (**a**), PL emission spectra for red CdTe/CdS QD-SpyCatcher and green CdTe/CdS QD-PilinC conjugates with $CsgA_{wt}$ amyloid fibers (black curve) or co-organized on $CsgA_{SpyTag}$ + $CsgA_{IsopeptagC}$ amyloid fibers (red curve) fabricated by living cells. EDS spectra, TEM, and HRTEM of the red-emission CdTe/CdS QD-SpyCatcher and green-emission CdTe/CdS QD-PilinC conjugates co-assembled on mixed $CsgA_{SpyTag}$ and $CsgA_{IsopeptagC}$ amyloid fibrils with living cells. (**b**) PL emission spectra for red CdTe/CdS QD-SpyCatcher, green CdTe/CdS QD-PilinC, and blue ZnCdSe/ZnS QD-PilinN conjugates with $CsgA_{wt}$ amyloid fibers (black curve) or co-assembled on $CsgA_{SpyTag}$ + $CsgA_{IsopeptagC}$+ $CsgA_{IsopeptagN}$ amyloid fibers (red curve) produced by living cells. EDS spectra, TEM, and HRTEM of the red CdTe/CdS QD-SpyCatcher, green CdTe/CdS QD-PilinC, and blue ZnCdSe/ZnS QD-PilinN conjugates co-organized with mixed $CsgA_{SpyTag}$ + $CsgA_{IsopeptagC}$+ $CsgA_{IsopeptagN}$ amyloid fibrils. TEM images repeated from main figure.

Additional References:

1 M. R. Chapman, L. S. Robinson, J. S. Pinkner, R. Roth, J. Heuser, M. Hammar, S. Normark and S. J. Hultgren, *Science*, 2002, **295**, 851-855.

2 A. Y. Chen, et al., , *Nature Mater.*, 2014, **13**, 515-523.

3 J. M. Callura, C. R. Cantor and J. J. Collins, *Proc. Natl. Acad. Sci. USA* 2012, **109**, 5850-5855.

4 R. Lutz and H. Bujard, *Nucleic acids research*, 1997, **25**, 1203-1210.

5 C. Prigent-Combaret, E. Brombacher, O. Vidal, A. Ambert, P. Lejeune, P. Landini and C. Dorel, *J. Bacteriol.*, 2001, **183**, 7213-7223.

6 B. Zakeri, J. O. Fierer, E. Celik, E. C. Chittock, U. Schwarz-Linek, V. T. Moy and M. Howarth, *Proc. Natl. Acad. Sci. USA*, 2012, **109**, E690-E697.

7 B. Zakeri and M. Howarth, *J. Am. Chem. Soc.*, 2010, **132**, 4526-4527.

8 Z. T. Deng, A. Samanta, J. Nangreave, H. Yan and Y. Liu, *J. Am. Chem. Soc.*, 2012, **134**, 17424-17427.

9 Z. T. Deng, O. Schulz, S. Lin, B. Q. Ding, X. W. Liu, X. X. Wei, R. Ros, H. Yan and Y. Liu, *J. Am. Chem. Soc.*, 2010, **132**, 5592-5593.

10 Y. G. Zheng, Z. C. Yang and J. Y. Ying, *Adv Mater*, 2007, **19**, 1475-1479.