Supporting Information

Chemo-Photothermal Therapy of Bacterial Infections by Metal-Organic Framework-Integrated Polymeric Network Coatings

Lulu Yu[1], Huajun Wu[1], Gnanasekar Sathishkumar[1], Xiaodong He [1], Runlong Ran[2], Kai Zhang[1*], Xi Rao[1], En-Tang Kang[1,3*], Liqun Xu[1*]

[1] BRICS Joint Laboratory on Biomedical Materials, School of Materials and Energy, Southwest University, Chongqing, P.R. China 400715

[2] College of Sericulture, Textile and Biomass Sciences, Southwest University, Chongqing, P.R. China 400715

[3] Department of Chemical and Biomolecular Engineering, National University of Singapore, Kent Ridge, Singapore 117576

***** To whom correspondence should be addressed:

E-mail: xulq@swu.edu.cn; cheket@nus.edu.sg; kaizhang095@swu.edu.cn

Experimental section

Instrumentation

Transmission electron microscopy (TEM) images were obtained from a FEI Talos F200S TEM equipped with Super X-EDS. UV−visible absorption spectroscopy was recorded on a Perkin Elmer Lambda 365+ UV/Vis spectrophotometer. X-ray photoelectron spectroscopy (XPS, Thermo Scientific ESCALAB 250Xi electron spectrometer) was used to analyze the surface elemental compositions. Nano ZS90 Zeta sizer (Malvern Instruments, UK) was used for the dynamic light scattering (DLS) and zeta potential (ZP) measurements. The surface morphology was observed by field-emission scanning electron microscopy (FESEM, JSM-7800F, JEOL Ltd., Tokyo, Japan) and scanning electron microscope (SEM, Phenom Pro, The Netherlands). The static WCA values of the substrates before and after modification were measured on a LR-SDC-350 goniometer using 3 μL DI water droplets. Ellipsometer (J.A.Woollam, Alpha-SE) was used to measure the thickness of the coating.

Synthesis of AuNRs

AuNRs was synthesized according to the classical seed-mediated growth method.[1] To prepare the gold seeds, 5 mL of 0.5 mM $HAuCl_4$ was mixed with 5 mL of 0.2 M CTAB solution in a-20 mL scintillation vial. Subsequently, 0.6 mL of fresh 0.01 M $NaBH_4$ was diluted with 1 mL of DI water and then injected into the Au(III)-CTAB solution under vigorous stirring (1,200 rpm). The color of the solution changed from yellow to brownish yellow. After stirring for 2 min, the seed solution was aged at room temperature for 2 h to exhaust the excess $NaBH_4$ before use.

To synthesize AuNRs with a LSPR peak at about 790 nm, 8.4 mL of $AgNO_3$ aqueous solution (4 mM) was introduced into 200 mL of CTAB aqueous solution (0.2 M). Then, the solution was mixed with 200 mL of $HAuCl_4$ aqueous solution (1 mM). After that, 2.8 mL of aqueous solution of ascorbic acid (78.8 mM) was added under stirring. The solution turned from yellow to colorless in a matter of seconds. Next, 0.48 mL of the prepared seed solution was introduced under stirring, and the solution was kept in a 37 °C water bath for 24 h. The resultant solution was centrifuged at 10,000 rpm for 20 min to precipitate the synthesized AuNRs. Finally, the prepared AuNRs were stored in 40 mL of DI water for subsequent use.

Ligand exchange of CTAB-stabilized AuNRs with PVP

The CTAB-stabilized AuNRs (40 mL) were centrifuged at 10,000 rpm for 20 min to precipitate the AuNRs. After removing the supernatant, 100 mL of PVP (6.7 g, M_w = 40,000) solution in

methanol was added into the AuNRs suspension. The mixture was sonicated into a homogeneous solution, followed by stirring at room temperature for 2 h. Then, the PVP-stabilized AuNRs were collected by centrifugation at 8,000 rpm for 20 min. The sample was redispersed in 40 mL of methanol for further use.

Synthesis of AuNR-ZIF-8 core-shell nanostructures

Four mL of the PVP-stabilized AuNRs and 8 mL of 2-MIM (14.25 mg) were mixed and stirred for 2 min at room temperature. Then, 8 mL of $Zn(NO_3)_2 \cdot 6H_2O$ (30.38 mg) solution in methanol was added to the mixed solution. After stirring at room temperature for 1 h, the final product was collected by centrifugation at 8,000 rpm for 10 min, washed with methanol twice, and dried at room temperature.

Drug loading efficiency of the AuNR-ZIF-CUR-2 nanostructures

CUR (100 mg) was dissolved in ethanol in a 10 mL volumetric flask to achieve a concentration of 10 mg/mL. The solution was diluted with ethanol to obtain a series of concentrations (10, 8, 6, 4, 2, and 0 μg/mL). The absorbance of the standard solution at 425 nm was measured on a Lambda 365+ UV/Vis Spectrophotometer, and the standard curve was plotted between the concentrations and absorbance. The dried AuNR-ZIF-CUR-2 sample was dispersed in ethanol, and its absorbance at 425 nm was measured. The drug loading capacity (DLC, %) of CUR was calculated using the following equation:

$$DLC(\%) = \frac{m_{CUR}\ in\ AuNR{-}ZIF{-}CUR{-}2}{m_{AuNR-ZIF-CUR-2}}$$

Release of CUR from the AZC-PA-Ply coating

The drug release behavior of the AZC-PA-Ply coating was investigated in PBS. The sample was irradiated with 808 nm NIR laser (0.5 W/cm^2) to achieve a temperature of 52 ^{o}C. For comparison, the sample was also immersed in PBS without the NIR irradiation. The amount of released CUR was quantified by measuring the OD value at 425 nm using a microplate reader at 20-minute time intervals.

Antioxidant activity of the AZC-PA-Ply coating

To determine the antioxidant potential, 5 μL of PBS (control) and samples-pretreated PBS were added into the wells of a 96-well plate. The plate was kept for 30 min, followed by the addition

of 180 μL of the working solution of ferric-reducing ability of plasma (FRAP) to each well. After 3-5 min of incubation at 37 ºC, the OD of each well was recorded at 570 nm in a microplate reader.

Cytokine secretion of macrophages

The pristine and modified PDMS substrates were directly placed into 1 mL of the cell culture medium for 48 h. The conditioning medium was then used to culture the Raw264.7 macrophages (1.0×10^4 cells) in a 24-well plate for 24 h. After co-culturing, the supernatant was collected and centrifuged. The expression levels of IL-6, IL-10, and TNF-α in Raw264.7 macrophages were determined by enzyme-linked immunosorbent assay (ELISA) Kits, according to the manufacturer's protocol.

Cytotoxicity of the AZC-PA-Ply coating

The cytotoxicity of AZC-PA-Ply coating towards L929 mouse fibroblast cells was evaluated by the CCK-8 assay. The samples were placed in a 24-well plate containing 1 mL of MEM. After 1, 3, and 5 days of immersion, the conditioning medium was used to culture the L929 cells for 24 h. Then, 10 μL of the CCK-8 solution was added to each well, and the plate was cultured at 37 ^{o}C for another 2 h. The OD of each well was recorded at 450 nm in a microplate reader.

Proteins and DNA leakage assays

Five hundred μL of bacterial suspension (1×10^7 cfu/mL) was incubated with different samples at 37 ^{o}C for 4 h. After co-incubation, the suspensions were centrifuged (4 ^{o}C, 10,000 rpm, 5 min) to collect the supernatant. The leakage of proteins was detected by an enhanced bicinchoninic acid (BCA) protein detection kit in a 96-well plate. For the leakage of DNA, the supernatant was filtered with 0.1 μm microporous membrane, and the absorbance of the purified sample at 260 nm was measured using a Lambda 365+ UV/Vis Spectrophotometer.

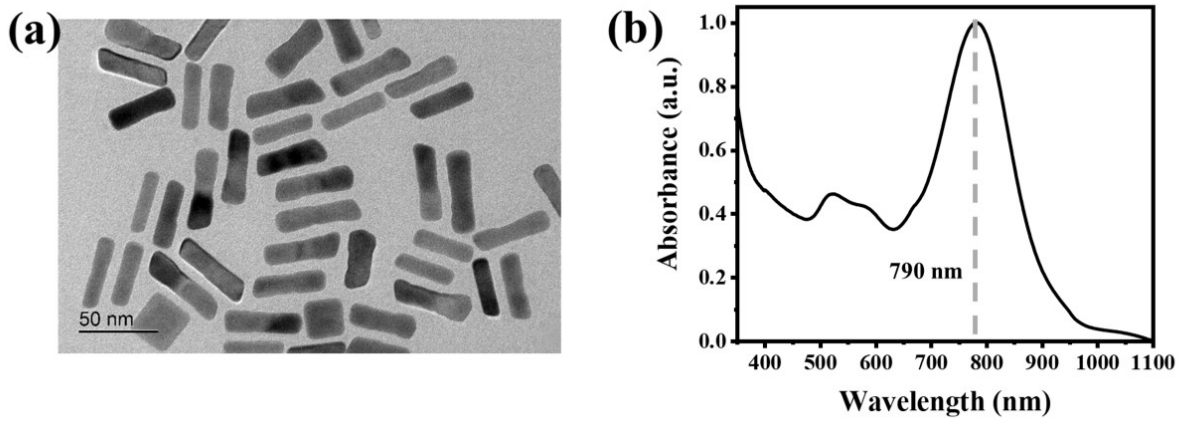

Figure S1. (a) TEM image of the CTAB-stabilized AuNRs. (b) UV−Vis−NIR absorption spectrum of the CTAB-stabilized AuNRs with a LSPR peak at ~ 790 nm.

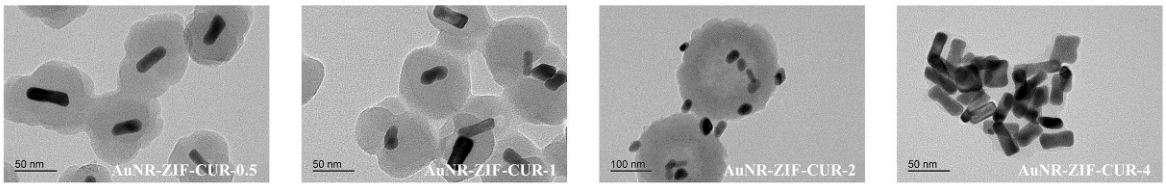

Figure S2. TEM images of the AuNR-ZIF-CUR-0.5, AuNR-ZIF-CUR-1, AuNR-ZIF-CUR-2, and AuNR-ZIF-CUR-4 composites.

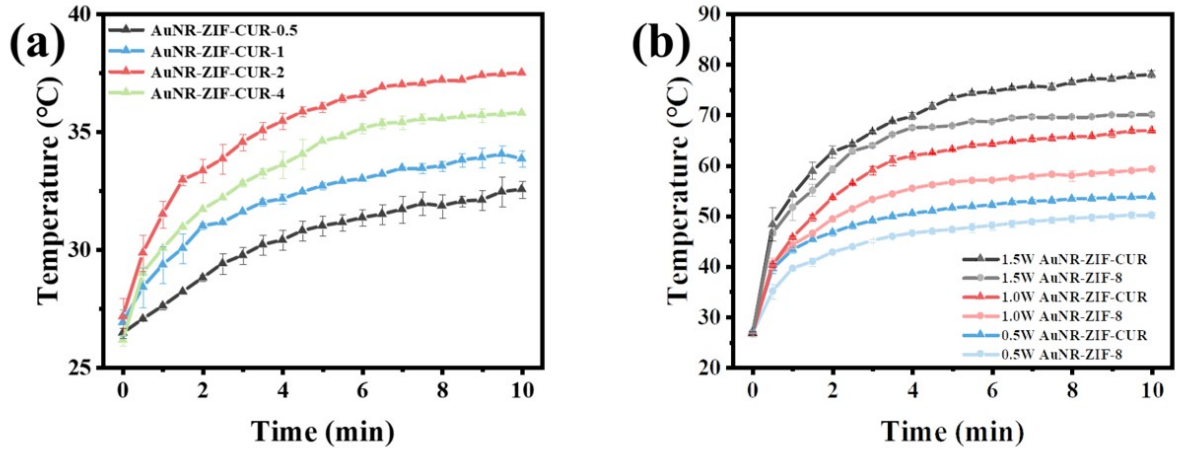

Figure S3. (a) Time-dependent temperature changes of Au-ZIF-CUR-0.5, Au-ZIF-CUR-1, Au-ZIF-CUR-2, and Au-ZIF-CUR-4 (0.1 mg/mL) in PBS under NIR (808 nm, 0.5 W/cm^2) irradiation. (b) Time-dependent temperature changes of AuNR-ZIF-8 and AuNR-ZIF-CUR (0.2 mg/mL) in PBS under NIR (808 nm, 0.5, 1.0, and 1.5 W/cm^2) irradiation.

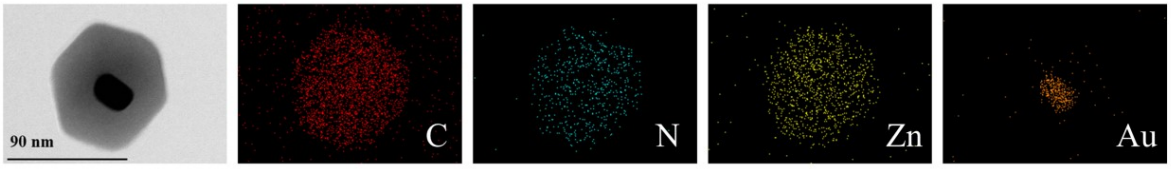

Figure S4. EDX elemental mapping of the AuNR-ZIF-8 core-shell nanostructure.

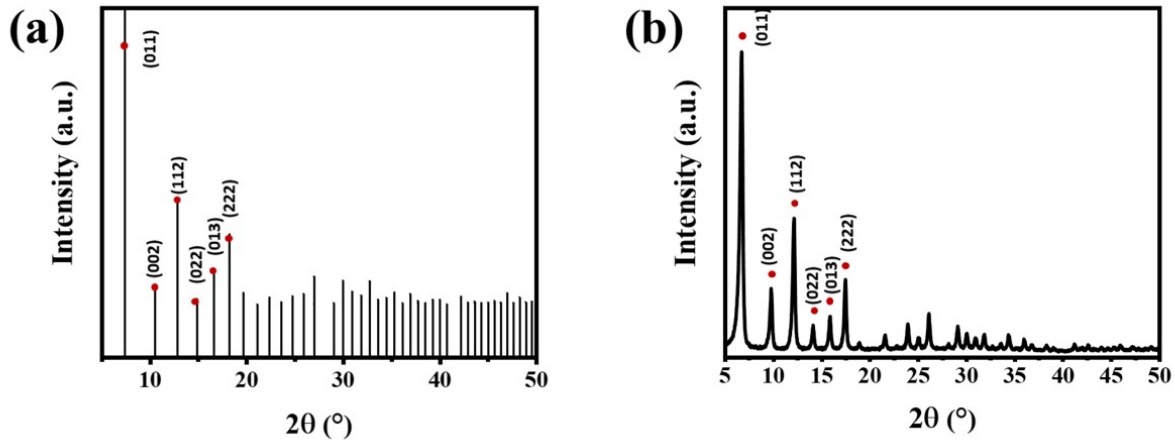

Figure S5. (a) XRD patterns of simulated ZIF-8 crystals and the synthesized ZIF-8 nanostructure.

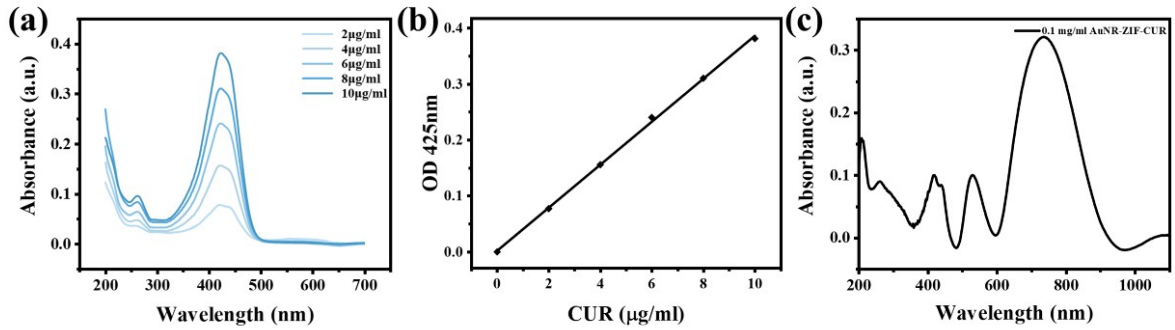

Figure S6. (a) UV−visible absorption spectra of CUR in methanol at different concentrations. (b) Linear relationship between the concentration of CUR and its absorption intensity (the linear range is 2 ~ 10 μg/mL with $R^2 = 0.9988$), and the regression equation is Y = 0.03843 X + 0.00206. (c) UV−visible−NIR absorption spectrum of AuNR-ZIF-CUR in methanol (0.1 mg/mL).

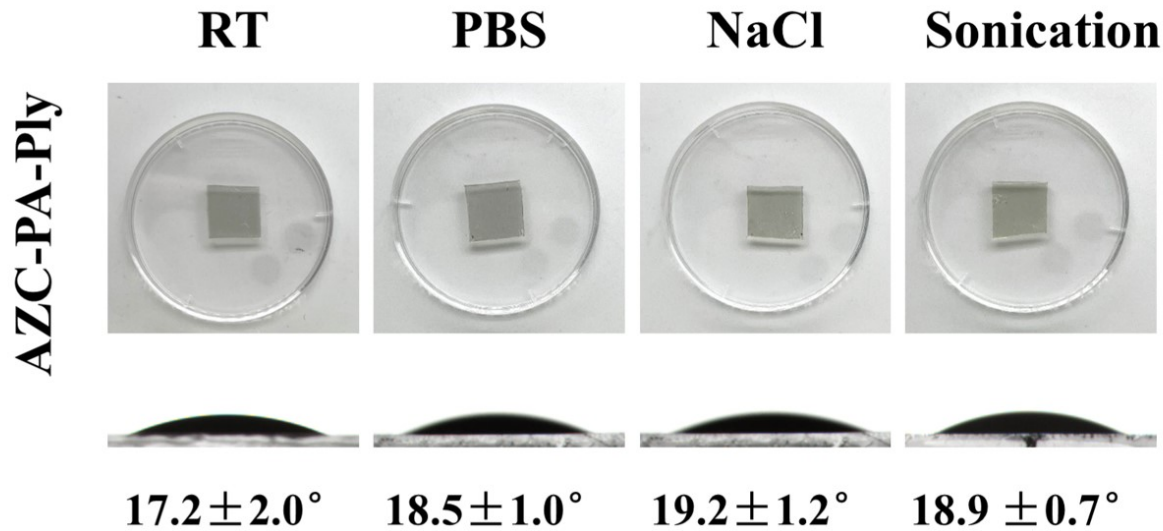

Figure S7. Photographs and static WCAs of the AZC-PA-Ply surfaces after standing at room temperature for 7 days and soaking in PBS and saline solution for 7 days, and sonicating for 7 min (100W).

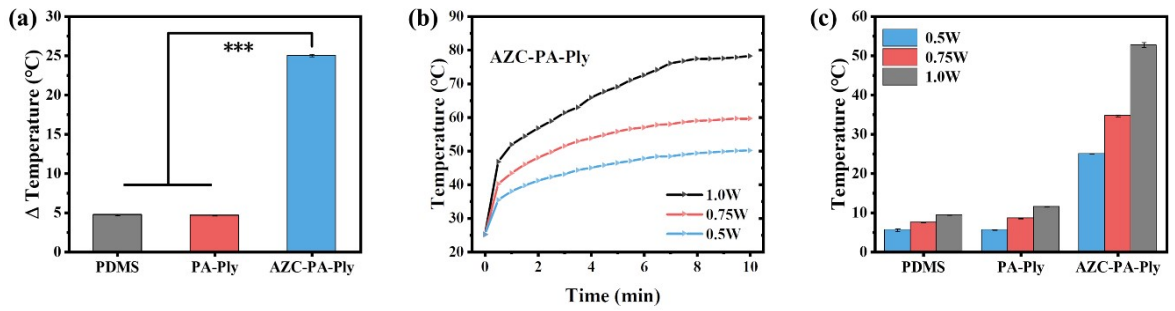

Figure S8. (a) ΔT of the pristine, PA-Ply-modified, and AZC-PA-Ply-modified PDMS surfaces. (b) Time-dependent temperature changes of the AZC-PA-Ply coating under NIR irradiation of different power intensities (808 nm, 0.5, 0.75, and 1.0 W/cm^2). (c) ΔT of the AZC-PA-Ply coating under NIR irradiation of different power intensities for 10 min.

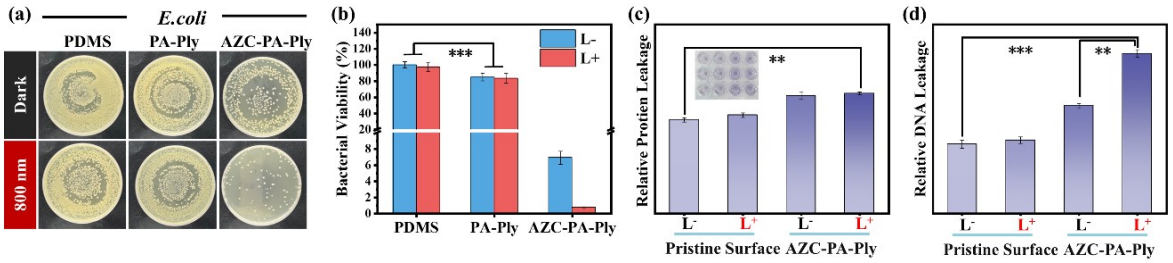

Figure S9. (a) Digital photos of TSB-agar plates inoculated with *E. coli* and (b) the number of *E. coli* detached from the pristine and modified PDMS surfaces in the presence (L^+) and absence (L^-) of NIR irradiation. (c) Protein and (d) DNA leakage of *E. coli* after different treatments. *** $p < 0.001$; ** $p < 0.01$.

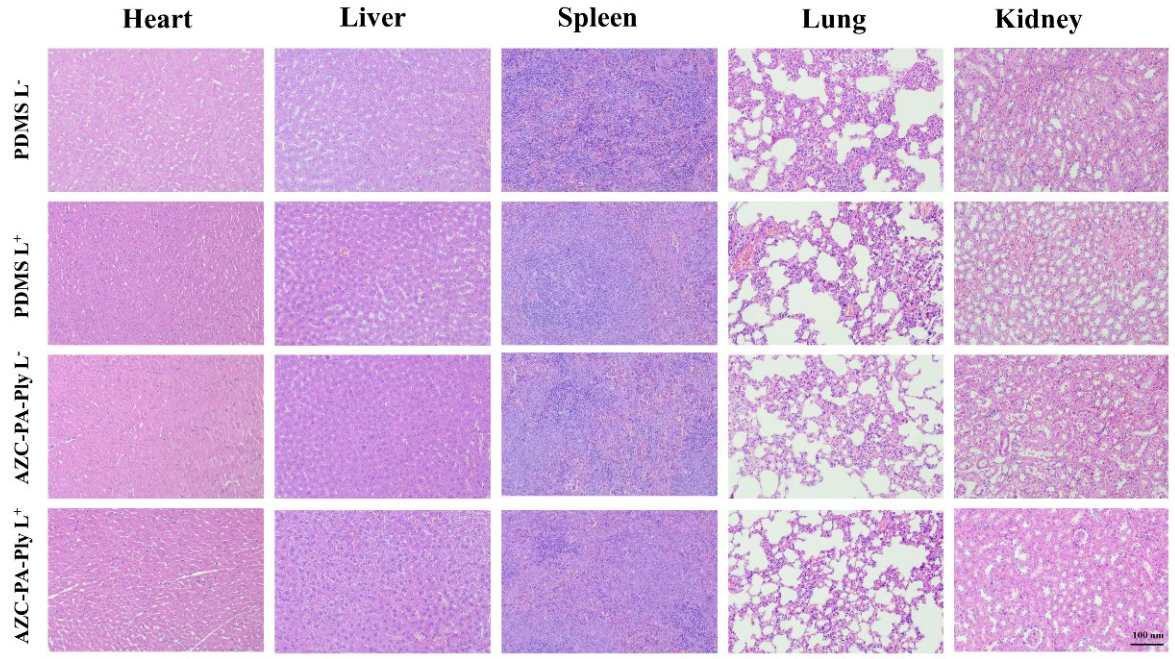

Figure S10. H&E staining images of the heart, liver, spleen, lung, and kidney of the healthy, as well as the pristine and modified PDMS-implanted, SD rats.

Reference:

1. Nikoobakht, B. and M.A. El-Sayed, *Preparation and Growth Mechanism of Gold Nanorods (NRs) Using Seed-Mediated Growth Method.* Chemistry of Materials, 2003. **15**(10): p. 1957-1962.