Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Honeycomb-Patterned Phthalocyanine Films with Photo-Active Antibacterial Activities

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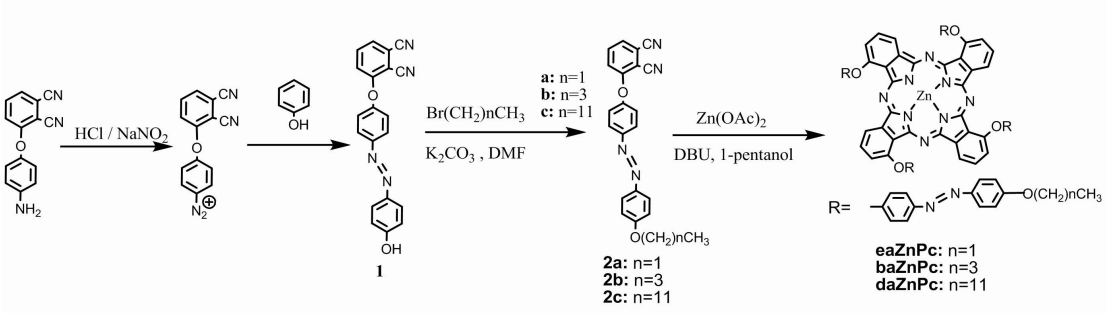
1. Synthesis of ZnPcs



Scheme S1. Synthetic route to azobenzene-substituted ZnPcs.

1. 1 Materials 4-Phenylazophenol was purchased from Alfa Aesar and used without further purification. The other reagents used were commercial products from Beijing Chemical Reagent Co. Solvents for spectroscopy were refined by standard methods, while others were used as received.

1. 2 Instruments NMR spectra were recorded on a JEOL JNM-ECA300 spectrometer with tetramethylsilane (TMS) as internal reference. Mass spectrometry was obtained on a Bruker LC-MS/MS (Esquire-LC) 1100 series; TOF-MS spectra were measured with BEFLEX III.

1. 3 Synthesis of 3-[4-(4-Dodecyloxy-phenylazo)-phenoxy]-phthalonitrile (compound 2c).

3-[4-(4-Hydroxy-phenylazo)-phenoxy]-phthalonitrile (compound 1) was synthesized according to the paper reported lately and used without certification.[1] 1 mmol compound 1 was dissolved in DMF (10 mL). 0.411 g finely powdered dry potassium carbonate and 1.494 g bromo-dodecane were added. The mixture was stirred at 70 ^{0}C for 3 h under argon protection. The final dark yellow suspension was cooled to ambient temperature and poured into 50 mL ice water, yielding much yellow precipitate. The precipitate was stirred in the NaOH aqueous solution with pH value of 14 and then filtered. Monitored by TLC chromatography, the product was used directly in the next step without further purification.

1. 4 Synthesis of daZnPc.

Compound 2c (0.30 g) was dissolved in pentanol (20 mL) with 1, 8-Diazabicyclo[5.4.0] undec-7-ene (DBU, 0.14 g) and $Zn(OAc)_2$ 0.04 g. The mixture was refluxed for 3 h under argon protection. The final green suspension was cooled to ambient temperature and poured into 40 mL methanol. The precipitate was filtered off. Finally, pure azo-ZnPc (112 mg) was obtained by chromatography on silica gel with commercially available chloroform as the eluent. The product was not soluble in DMSO. Chemical analysis: MS (MALDITOF, THF) m/z: 2100.00, 1H NMR (300 MHz, d6-$CHCl_3$) ppm: 7.26—7.91 (m, 32H, ArH), 6.97—7.26(m, 12H, ArH), 4.04—4.06 (m, 2H, CH2), 1.29—1.86 (m, 14H, alkyl-H), 0.85—0.89(m, 9H, alkyl-H).

1. 5 Synthesis of 3-[4-(4-Ethoxy-phenylazo)-phenoxy]-phthalonitrile (compound 2a).

0. 400 g compound 1 was dissolved in DMF (10 mL). 0. 484 g finely powdered dry potassium carbonate and 0.80 g bromoethane were added. The mixture was stirred at 70 ^{0}C for 3 h under argon protection. The final dark yellow suspension was cooled to ambient temperature and poured into 50 mL ice water, yielding much yellow precipitate. The precipitate was stirred in the NaOH aqueous solution with pH value of 14 and then filtered. Monitored by TLC chromatography, the product was used directly in the next step without further purification.

1. 6 Synthesis of eaZnPc.

3-[4-(4-Ethoxy-phenylazo)-phenoxy]-phthalonitrile (0.30 g) was dissolved in pentanol (20 ml) with 1, 8-Diazabicyclo[5.4.0] undec-7-ene (DBU, 0.22 g) and $Zn(OAc)_2$ 0.06 g. The mixture was refluxed for 3 h under argon protection. The final green suspension was cooled to ambient temperature and poured into 40 mL methanol. The precipitate was filtered off. Finally, pure Et-O-Pc (104 mg) was obtained by chromatography on silica gel with commercially available chloroform as the eluent. Chemical analysis: MS (MALDITOF, THF) m/z: 1537.4, 1H NMR (300 MHz, d6-DMSO) ppm: 6.93—7.91 (m, 44H, ArH), 4.07—4.12 (m, 2H, CH2), 1.31—1.41 (m, 3H, alkyl-H).

1. 7 Synthesis of 3-[4-(4-Ethoxy-phenylazo)-phenoxy]-phthalonitrile (compound 2b).

0.873 g compound 1 was dissolved in DMF (10 mL). 1.400 g finely powdered dry potassium carbonate and 2.133 g 1-bromobutane were added. The mixture was stirred at 70 ^{0}C for 3 h under argon protection. The final dark yellow suspension was cooled to ambient temperature and poured into 50 mL ice water, yielding much yellow precipitate. The precipitate was stirred in the NaOH aqueous solution with pH value of 14 and then filtered. Monitored by TLC chromatography, the product was used directly in the next step without further purification.

1. 8 Synthesis of baZnPc.

3-[4-(4-Butoxy-phenylazo)-phenoxy]-phthalonitrile (0.40 g) was dissolved in pentanol (20 ml) with 1, 8-Diazabicyclo[5.4.0] undec-7-ene (DBU, 0.22 g) and $Zn(OAc)_2$ 0.28 g. The mixture was refluxed for 3 h under argon protection. The final green suspension was cooled to ambient temperature and poured into 40 mL methanol. The precipitate was filtered off. Finally, pure Bu-O-Pc (192 mg) was obtained by chromatography on silica gel with commercially available chloroform as the eluent. Chemical analysis: MS (MALDITOF, THF) m/z: 1649.5, 1H NMR (300

MHz, d6-DMSO) ppm: 6.94—8.29 (m, 44H, ArH), 4.01—4.05 (m, 2H, CH2), 1.71—1.75 (m, 2H, CH2), 1.42—1.47 (m, 2H, CH2), 0.91—0.97 (m, 3H, CH3).

2. J-aggregation of Pcs in the solution

2. 1 Instruments Absorption spectra were measured on a HP8452A spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer. UV-illumination was carried out with a high-pressure mercury lamp (mejiro precision, SHG-200; 1000 W, made in Japan) with suitable filters. The concentration of the ZnPc chloroform solutions are 4.0×10^{-6} M, if not otherwise indicated.

2. 2 J-aggregation of daZnPc

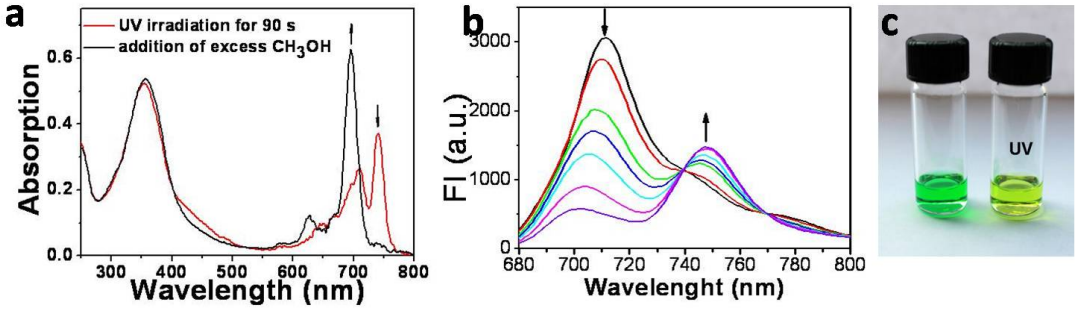


Fig. S1 (a) Changes in the absorption spectra of daZnPc in $CHCl_3$ upon addition of methanol. Since methanol coordinates with daZnPcs, the titration of methanol redistributed J-type aggregates into monomers. (b) Fluorescence intensity changes of daZnPc chloroform solution upon irradiation with 365 nm UV light. (c) The solution color change from green to green yellow after UV illumination.

2. 3 J-aggregation of eaZnPc

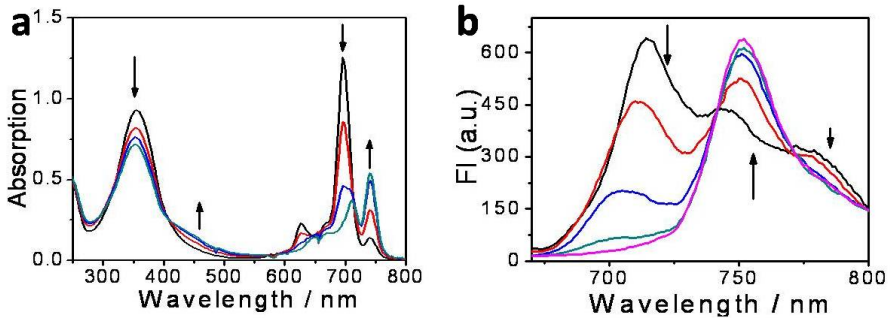


Fig. S2 (a) Absorption spectral changes of eaZnPc in chloroform under the irradiation of 365 nm UV light. (b) Fluorescence intensity changes of eaZnPc chloroform solution upon irradiation with 365 nm UV light.

2. 4 J-aggregation of baZnPc

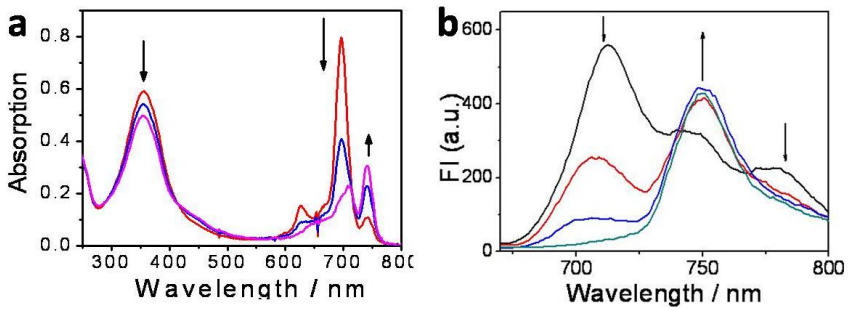


Fig. S3 (a) Absorption spectral changes of baZnPc in chloroform under the irradiation of 365 nm UV light. (b) Fluorescence intensity changes of baZnPc chloroform solution upon irradiation with 365 nm UV light.

2. 5 Photo-modulation dynamics of J-aggregation

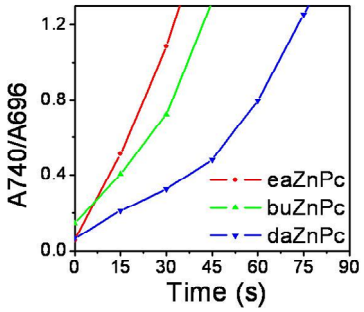


Fig. S4 Time-dependent absorption spectral changes of A740/A696 of ZnPcs in chloroform under the irradiation of 365 nm UV light. The J-aggregates in daZnPc solution formed at a much lower pace, though similar amount of J-aggregates can be obtained.

3. Honeycomb patterns formation

3. 1 Methods and Instruments. To prepare TEM and AFM samples for the images of the aggregation, a drop of daZnPc chloroform solution (8.0×10^{-6} M) with different time of UV light illumination was deposited onto substrates at room temperature. The relative humidity (RH) is 25%, if not otherwise indicated. Transmission electron microscopy (TEM) was performed on a model H-800 electron microscope operating at acceleration voltage of 100 kV. AFM Atomic force microscopy (AFM) images were taken at room temperature in air tapping mode on a commercial instrument Nanoscope IV (Veeco Company, USA.) using Silicon cantilevers (200 - 300 kHz, Veeco Company, U.S.A.). Silicon wafers were purchased from Wafer Net GmbH, Germany, and treated with piranha (7:3 v:v of sulfuric acid and 30% hydrogen peroxide), and then washed with water and dried with high pure nitrogen flow to get a hydrophilic surface. The freshly prepared hydrophilic silicon wafers were incubated in the deionized water and dried in air right before use.

3. 2 AFM imaging of daZnPc honeycomb structures

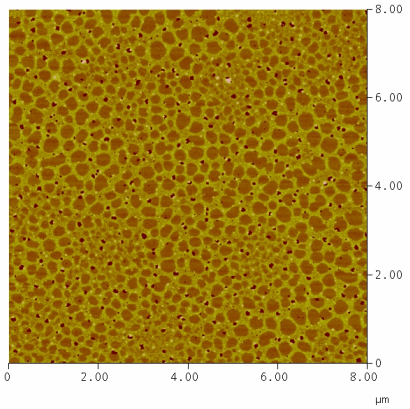


Fig. S5 The typical AFM image of the honeycomb-patterned Pc film showed the uniformity of the BF structures.

3. 3 Impact of Relative humidity (RH). The influence of RH on the formation of daZnPc honeycomb-pattern was studied (Fig. S6). When RH was decreased to 1%, only donut-like aggregates with average diameter of 200 nm formed, because there was not water vapor in the environment to condense as templates. When RH was over 80%, the honeycomb structure was enlarged to micrometer scale and the uniformity was deteriorated, implying the coalescence of water templates. These results indicated that the small molecule daZnPc can form honeycomb-pattern by BF technique at proper RH: the water condensed from the air and self-assembled at the air-solution interface when chloroform evaporated, serving as templates for J-aggregated daZnPc to solidify around, resulting in honeycomb patterns.

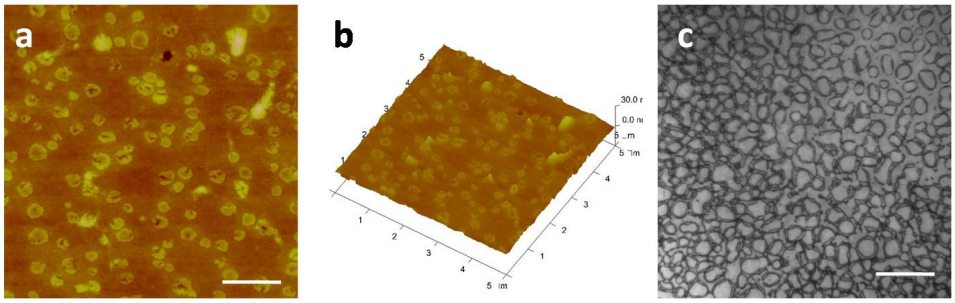


Fig. S6 (a) AFM image of daZnPc chloroform solution at RH <1%. Scale bar: 1μm. (b) 3D AFM image showed the donut-like morphology of daZnPc aggregates at RH <1%. (c) Optical microscopy image showed irregular honeycomb structures when RH was 80% at room temperature. Scale bar: 3 μm.

3. 4 Impact of alkoxy chain length. eaZnPc and baZnPc could not form ordered honeycomb pattern as daZnPc did (Fig. S7), though they had similar J-aggregation process in solution. Thus, the lengthy aliphatic chain was important for the daZnPc honeycomb-pattern formation due to the enhanced J-aggregation at interface by van der Waals interaction, as well as the increased solubility and flexibility of the aggregates, which inhibited crystallization and facilitated the interaction between the daZnPc and water templates in forming honeycomb-patterned films.

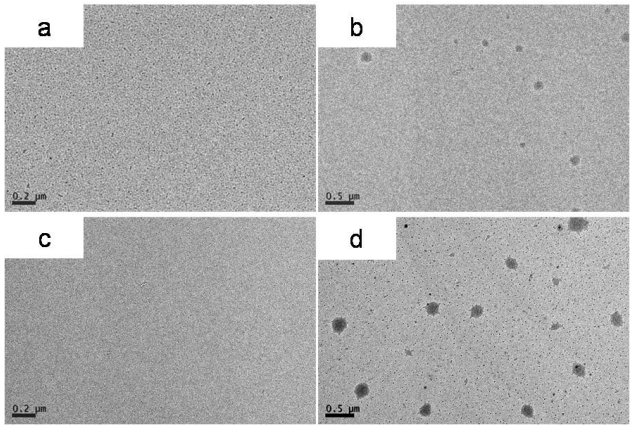


Fig. S7 TEM images of samples prepared from (a) eaZnPc chloroform solution without UV illumination and (b) eaZnPc chloroform solution with enough UV illumination to complete J-aggregation; (c) baZnPc chloroform solution without UV illumination and (b) baZnPc chloroform solution with enough UV illumination to complete J-aggregation.

4. Singlet oxygen generation

The Pc films used in this section were fabricated on glass slides at 25% RH at room temperature. All the chloroform solution had the same concentration of c = 4.0×10^{-6} M. daZnPc films were fabricated using fully-illuminated daZnPc solution in which sufficient J-aggregation was obtained. Drop-cast daZnPc film was fabricated by BF technique used above and confirmed that regular honeycomb patterns can also be formed on glass slide except for silicon wafer. Spin-coat daZnPc film was fabricated by spin-coating at 500 rpm for 2 min (Fig. S8b). Drop-cast ZnPc film was fabricated by the same method as drop-cast daZnPc film (Fig. S8a). All of the films were freshly fabricated before use in the quenching studies and antibacterial test below. The AFM images of the films were shown in Fig. S8.

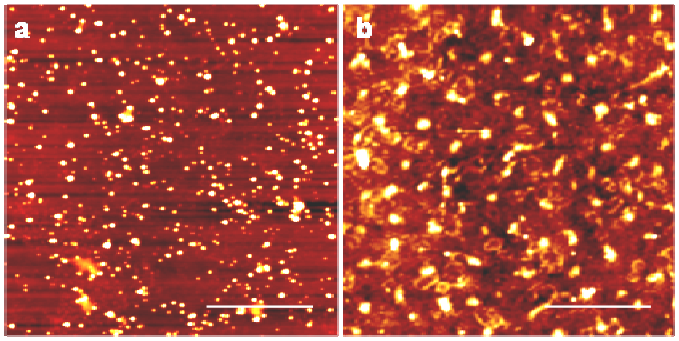


Fig. S8 AFM images of (a) drop-cast ZnPc film (H-aggregates) (b) spin-coated daZnPc film (no honeycomb pattern found). Scale bar: 1 μm. RH = 25 %, r.t.

SOSG was purchased from Invitrogen and a 5 mM stock solution in methanol was prepared. ZnPc and sodium azide (NaN_3) were purchased from Sigma-Aldrich. A 5 μM SOSG working solution was made by dissolving SOSG in phosphate buffered saline (PBS) solution which had been bubbled with oxygen previously before for 30 min. 1 mL of SOSG solution was placed in a

cell of 6-well plate with different films and then irradiated by 0.4 mw/cm^2 halogen lamp ((Fiber-Lite® High Intensity Illuminator, MI-152, Dolan-Jenner. Industries, Inc., a > 600 nm long-pass filter was reduce the self-fluorescence of SOSG). The resulting fluorescence signal was read-out using a BioTek Synergy H4 Hybrid reader (excitation/emission: 504/525 nm). Sodium azide was added to the working solution (10 mM) and used as a fluorescence-quenching control.

5. Antibacterial effect

5. 1 Methods and Instruments. The bactericidal activity of the Pc films was tested using amoxicillin-resistant *Escherichia coli* (*E. coli*) and PBS solution (containing 1/1000 amoxicillin). Inoculations were given from the fresh colonies on agar plates into 5 mL LB solution. Cell growth was allowed by shaking in the oven at 37°C overnight. Then, *E. coli* cells (a 0.5 value of OD_{600} featuring 1×10^7 CFU/mL) was centrifuged at 10 000 rpm, washed by PBS solution two times and then resuspended in PBS solution. The 0.4 mw/cm^2 halogen lamp was used without filter. All the experiments were carried out in triplicate (i.e., three samples for each test) and repeated at least three times.

5. 2 Contact bactericidal activities in PBS. 200 μL of PBS solution containing ~ 1×10^6 *E. coli* cells was deposited on the films and then irradiated with 0.4 mw/cm^2 white light for 3 hours. After the experiment, the *E. coli* solution was diluted several times using PBS solution and 10 μL was spread uniformly over the LB agar plate (Fig. S9a-b). The Petri dishes were incubated at 37°C for 24 hours for colonies formed can be observed clearly by naked eyes (Fig. S9c). The antibacterial effectiveness of the samples was calculated using the following formula: R (%) = (B-C)/B × 100, where R is the cell death (%), B is the CFU number of bacteria in the presence of glass slide, and C is the CFU number of bacteria in the presence of daZnPc film.

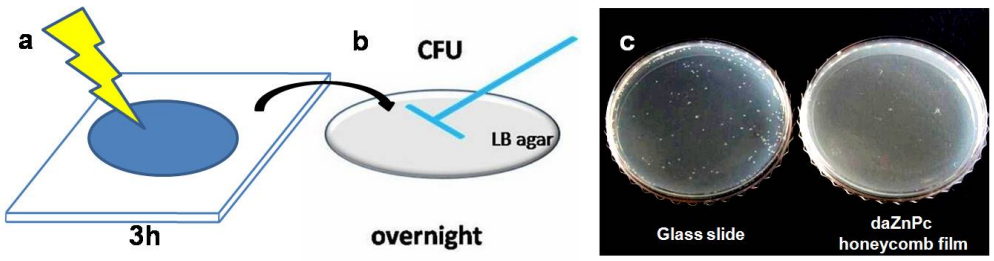


Fig. S9 Demonstration of the CFU assays procedure: (a) The cell culture solution with honeycomb daZnPc film was illuminated by white light for 3 hrs. (b) The supernatant solution was transferred to LB agar plate for overnight cell growth. (c) The CFU formed on silicon wafer (blank control) and daZnPc honeycomb film, showing high antibacterial activity of the daZnPc film.

For SEM, the bacteria solutions were treated with a fixative solution of 2.5% glutaraldehyde. After ethanol dehydration, the solutions were drop-cast onto aluminum SEM stubs, and viewed with a FEI Nova NanoSEM 230 scanning electron microscope at an acceleration voltage of 3 KeV.

[1] a) L. H. Niu, C. Zhong, Z. H. Chen, Z. Zhang, Z. Y. Li, F. S. Zhang and Y. W. Tang, *Chinese Science Bulletin* **2009**, *54*, 1169-1175; b) Z. Chen, C. Zhong, Z. Zhang, Z. Li, L. Niu, Y. Bin and F. Zhang, *J Phys Chem B* **2008**, *112*, 7387-7394.