Electronic Supplementary Information

Mesoscopic organic nanosheets peeled from stacked 2D covalent frameworks

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1. General Information

All solvents and chemicals were used as obtained from commercial suppliers, unless otherwise stated. Dry solvents and nitrogen glove box were used for the set up of reactions. Photoacoustic Fourier-transform infrared (PA-FTIR) spectra were recorded on Digilab FTS 7000 FTIR spectrometer equipped with a MTEC-300 photoacoustic detector. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker AV-400 (400 MHz) spectrometer. Chemical shifts were reported in ppm from tetramethylsilane with the solvent resonance as the internal standard. Scanning electron microscopy (SEM) images were obtained on a JEOL JSM-7400F electron microscope (10 kV). TEM experiments were conducted on a FEI Tecnai G^2 F20 electron microscope (200 kV). Absorption spectra were recorded at room temperature on an Agilent 8453 ultraviolet-visible (UV-Vis) spectrometer, and fluorescence spectra were recorded at room temperature on a Jobin Yvon Horiba Fluorolog spectrometer. The fluorescence images were obtained with an Olympus Fluoview 300 confocal laser scanning system.

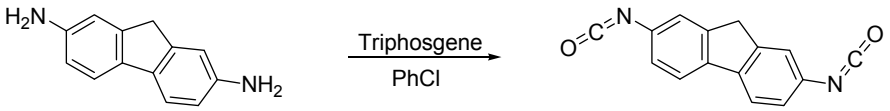
For AFM characterization, a 20-μL drop of **NS-Aa2** solution in methanol was deposited on a cleaned silicon dioxide substrate (1 cm × 1 cm) and dried in air. A commercial AFM instrument (Dimension 3100 with Nanoscope IIIa controller, Veeco Instruments Inc.,

CA) equipped with a scanner (90 µm × 90 µm) was used to image the samples in the tapping mode in air. Silicon cantilevers with the normal resonance frequency of 300 kHz and spring constants of 40 N/m (Tap300Al, Budget Sensors, Bulgaria) were used. All images were captured with a scan rate of 1–2 Hz and with a pixel resolution of 512 × 512.

2. Synthesis of PICU-A

In a glove box, **A** (980 mg, 5 mmol) was dissolved in DMF (50 ml) in a pressure flask, and 1,3-bis-*t*-butyl-4,5-dihydroimidazol-2-ylidene (SI^{t}Bu) (0.1 mmol, generated *in situ*) was added. The reaction flask was sealed, and placed in the oven at 80°C for 3 days. **PICU-A** was collected by filtration, washed with DMF, CH_2Cl_2 and ether, and dried in a vacuum oven. Quantitative yield of **PICU-A** was obtained.

3. Synthesis of 2,7-Diisocyanate Fluorene



Triphosgene (98.9 mg, 0.66 eq) was dissolved in a minimal amount (5 ml) of PhCl and heated to 80°C. 2,7-diaminofluorene (0.5 mmol) was separately dissolved in ~ 15 ml of PhCl with 2 eq. of Et_3N. Fluorene was then added dropwise to triphosgene and stirred for 30 min at 80°C. The reaction mixture was then heated to 130°C for 2 h. The reaction was cooled, diluted with CH_2Cl_2, and washed with deionized water 3 times. The yellow organic layer was dried over sodium sulfate and concentrated *in vacuo* to obtain a yellow solid. Yield = 75.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.83 (s, 2H, CH_2), 7.10 (dd, J 8 Hz; 2 Hz, 2H, C3), 7.24 (d, J 2 Hz, 2H, C1), 7.64 (d, J 8 Hz, 2H, C4). ^{13}C NMR (100 MHz, $CDCl_3$) δ 36.6, 120.5, 121.5, 123.7, 131.8, 138.5, 144.5. MS (LCMS) m/z 249 ($M + H^+$).

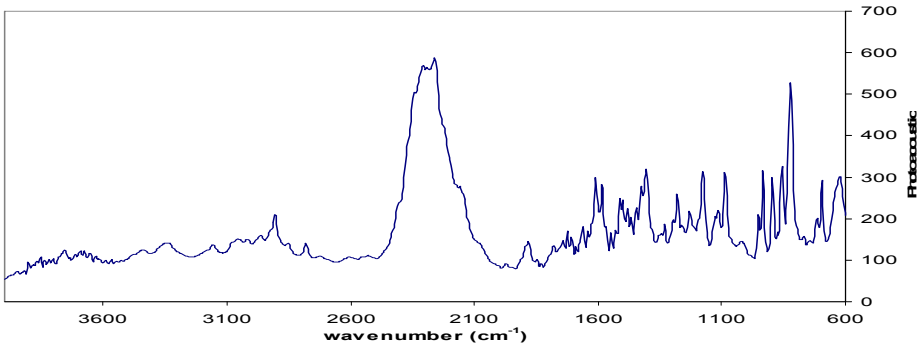


Figure S1. PA-FTIR spectrum of 2,7-diisocyanate fluorene.

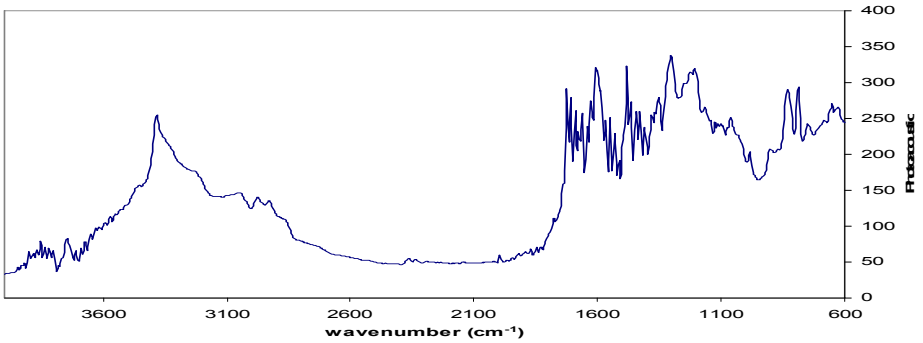


Figure S2. PA-FTIR spectrum of **PICU-B**.

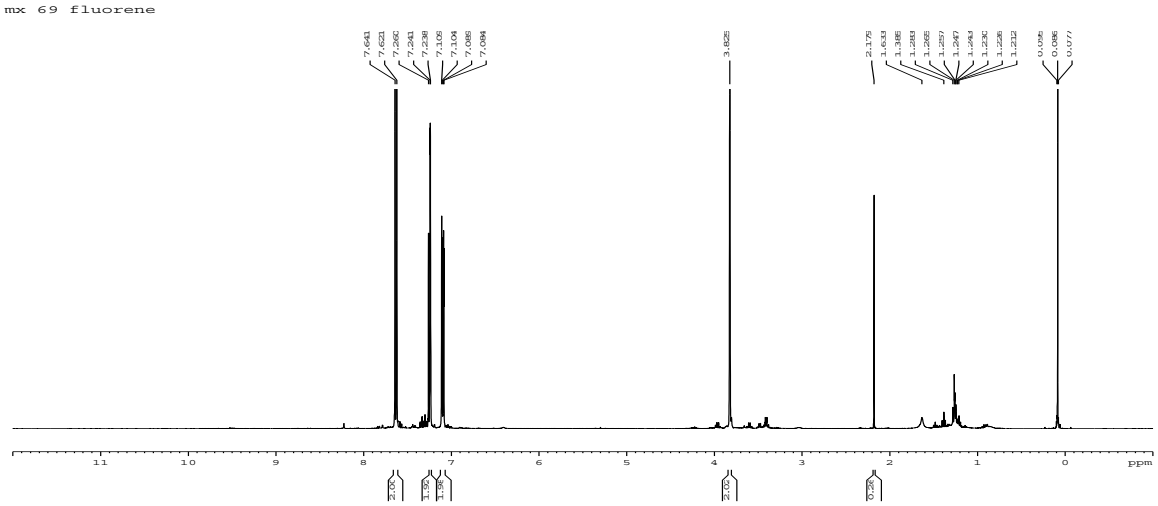


Figure S3. ^{1}H NMR spectrum of 2,7-diisocyanate fluorene.

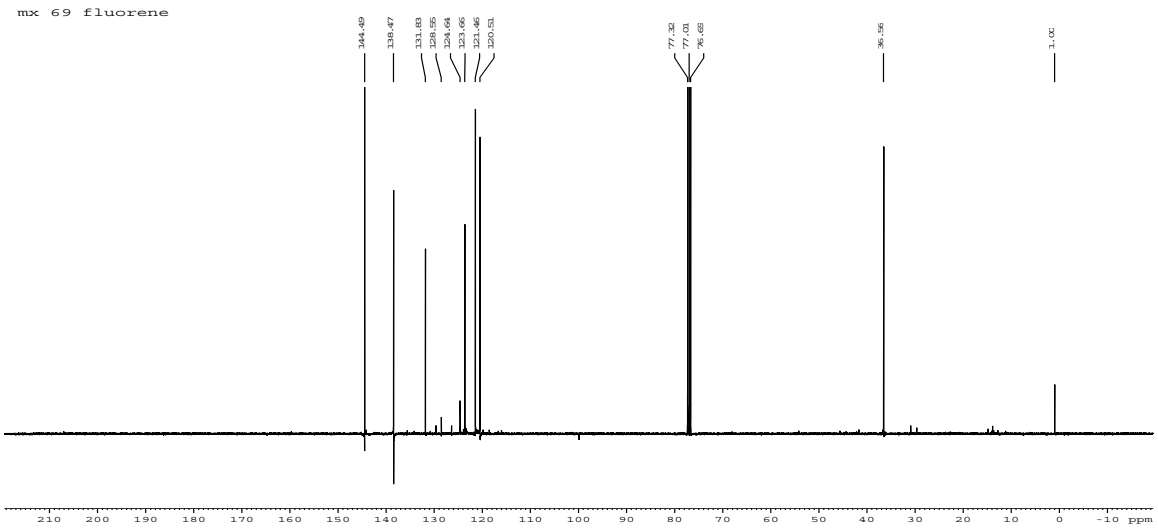


Figure S4. ^{13}C NMR spectrum of 2,7-diisocyanate fluorene.

4. Synthesis of Nanosheets

10 mg of **PICU-A** and 0.2 mmol of 3-aminopropanol (12 mg) were added to a reaction vial with 10 ml of DMF. The vial was capped and heated at 100°C for 48 h with stirring. The original **PICU-A** suspension was converted into a red solution. After removal of a small amount of unreacted solid via filtration, the filtrate was precipitated and washed with diethyl ether. **NS-Aa** was collected as an orange powder.

20 mg of **PICU-A** and 200 mg of PEG-NH_2 (Mw = 500) were mixed in a reaction vial with 20 ml of DMF. The vial was capped and heated at 80°C for 24 h with stirring to form nanosheets. After filtration, the unreacted solid was washed with DMF, and the filtrate was kept for analysis. The filtrate was precipitated and washed with diethyl ether. **NS-Ab** was collected as an orange powder.

10 mg of **PICU-A** and 10 mg of glucosamine were mixed in a reaction vial with 10 ml of DMF. The vial was capped and heated at 100°C for 72 h with stirring to form nanosheets. After removal of the unreacted solid by filtration, the filtrate was precipitated and washed with diethyl ether. **NS-Ac** was collected as a reddish-orange solid.

5. Preparation of Samples for Spectroscopic Analysis

NS-Aa (or **NS-Ba**) was dissolved in water/dimethyl sulfoxide (DMSO) (volume ratio = 10:1) at a concentration of 0.025 mg/ml. The solution was sonicated and used for UV spectroscopic analysis. The resulting solution was diluted 50-fold for fluorescence spectroscopic analysis.

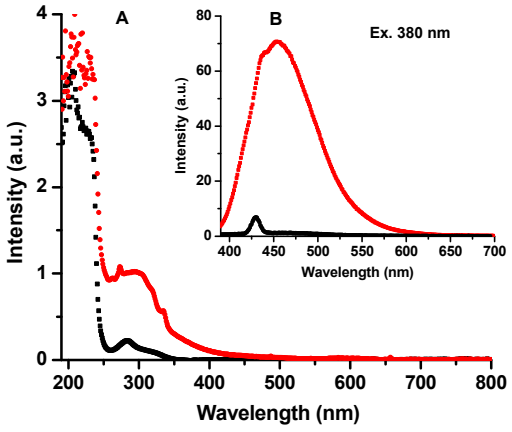


Figure S5. (A) Absorption and (B) emission spectra of **NS-Aa** (red) and **NS-Ba** (black).

6. NS-Ac Stock Solutions for Biological Applications

Stock solution **A** of **NS-Ac** was prepared by dissolving **NS-Ac** in water (1 mg/ml). The final concentration of **NS-Ac** in the culture medium was kept below 30 μg/ml. Stock solution **B** of Nile red/**NS-Ac** was prepared by adding 1 mg of Nile red to 5 ml of solution **A**. Nile red was purchased from Sigma, and used directly without further purification. The mixture was sonicated for 30 min, followed by filtration. An aqueous solution of Nile red (control) was prepared under the same condition by using water instead of stock solution **A**. RAW 264.7 cells or KB cells were trypsinized and resuspended in RPMI 1640 medium with 10% of fetal bovine serum (FBS) and 1% of penicillin/streptomycin. The cells were initially seeded in cell culture plates in 200 mL of full RPMI culture medium, and kept at 37°C and 5% of CO_2 for 48 h before the addition of compounds.

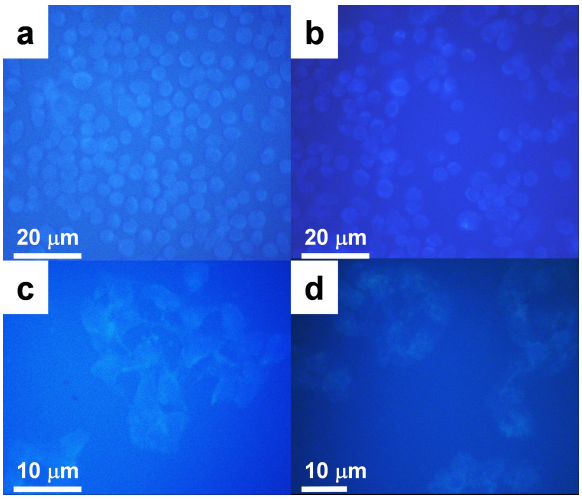


Figure S6. Confocal microscopy images of (a,b) RAW 264.7 and (c,d) KB cells incubated with **NS-Ac** for (a,c) 24 h and (b,d) 48 h.