

Supplementary Information

Soluble and highly fluorescent conjugated polymer network: nonoxidative reversible doping, cell imaging and anticancer activity

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Experimental

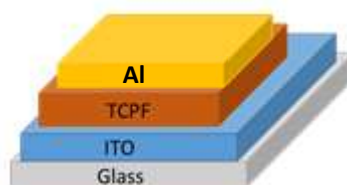
Materials

All chemicals were bought from Sigma-Aldrich Pvt. Ltd., including N-bromosuccinimide (NBS), 2-bromo-3-hexyl thiophene, diethylene glycol monoethyl ether, triethyl amine, p-Toluenesulfonyl chloride (TsCl), trifluoromethanesulfonic acid, (4-cyanophenyl)boronic acid etc. Other solvents obtained from Rankem included THF, ether, methanol, chloroform, dichloromethane, toluene, DMF, hexane, ethylacetate and others. THF was dried over sodium and benzophenone in an inert environment and employed for reactions right away. All other solvents like dichloromethane (DCM), toluene was dried by distillation before use.

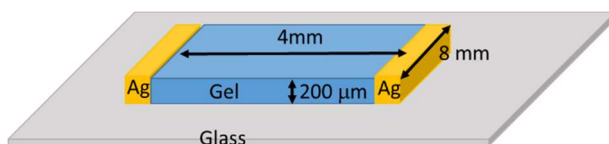
Characterization

Nuclear magnetic resonance (NMR) spectra were recorded on 500 MHz JEOL instrument. UV-vis spectra in solution and thin film state were recorded at room temperature using a UV-vis

spectrometer (Hewlett-Packard, model 8453). At room temperature, photoluminescence data in solution and thin film states were collected using a HORIBA Jobin Yvon (Fluoromax-3) luminescence spectrometer. Fluorolog-HORIBA was used for measuring absolute quantum yield and solid state emission studies. Cyclic voltammetry (CV) was performed in a Metrohm Autolab Electrochemical Analyzer using a standard three-electrode electrochemical cell in a 0.1 M tetrabutylammonium hexafluorophosphate solution in CH_2Cl_2 at room temperature in a nitrogen atmosphere at a scanning rate of 100 mVs^{-1} . The working electrode, counter electrode and reference electrode used are glassy carbon, Pt wire and silver wire respectively. For CV measurement, 2 mg/10 ml polymer concentration was used. Gel-permeation chromatography (GPC) was carried out on a Malvern Panalytical (UK) Model-GPC 5140 instrument. The molecular weight of the samples was determined using THF as an eluent (1 ml/min) at 35°C and polystyrene as a standard. AFM imaging of the thin film deposited on silicon substrate was performed using AFM NTMDT, SolverNext in the tapping mode. Analysis of the AFM images was performed with the WSxM software (Nanotec Electronica). Gel of TCPN was prepared by adding $100 \mu\text{l}$ of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ to THF solution of polymer (2 mg/ml). The solution changes color from greenish to light red, become viscous gradually and turns into gel within few hours. I–V measurements were carried out from the sandwich structures on ITO-coated glass substrate. Thin films ($\sim 400 \text{ nm}$) were spin coated from toluene solution of CTF (20 mg/ml) on thoroughly cleaned ITO pattern (width 3.5 mm) glass substrate. The samples were dried at 60°C for an additional 3 hrs in a vacuum oven for complete removal of the solvent. A layer of Al (width 2.5 mm) was deposited on the thin film to act as the top electrode. I–V characteristics of the samples were measured using a Keithley Source Meter Model 2450 SMU under inert condition. Schematic diagram for the device structure is shown below.



For I-V measurement, gel sample was placed on the glass substrate. Then two side of the gel sample were silver pasted and used as electrode. The distance between the two electrodes was kept at ~ 4mm, whereas the thickness and the width of the sample were ~200 μm and ~ 8 mm respectively. Schematic diagram for the device structure is shown as below.



Electrical conductivities were measured using a interdigitated gold electrode of 10 μm wide and 5 mm long with an interdigit distance of 50 μm , patterned on thermally grown silicon oxide. The substrates were cleaned by sonication in isopropanol and acetone, followed by ozone plasma cleaning. TCPN films were deposited by spin-coating from 20 mg/ml in toluene with speed 1500 rpm. I-V characteristics were measured using a multimeter. The film thicknesses were measured by AFM. The equation used for measuring conductivity is

$$\kappa = \frac{l}{Rwd}$$

Where R is the resistance of the sample measured at 8 Volt, l is channel length, w is length of the electrode and d is the thickness of the film. Gel conductivity was measured similarly from the device used for IV measurement described previous section.

Quantum chemical calculations:

To understand the effect of doping on the band gap we have performed quantum chemical calculations at the B3LYP/6-31G(d,p) level of theory using G09 suit of programs. We have

performed geometry optimization along with frequency calculations to make sure of stationary point geometry.

Cell Culture and maintenance: SiHa cells (cancerous tissues of the cervix uteri) were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% heat inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin. The temperature of the culture was maintained at 37°C in a CO₂ incubator with 5% CO₂ supply.

2.5 Cell viability The cell viability percentage was evaluated by using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT assay. Cell seeding was done in each well at the confluency of 70-80 % cells per well in 0.1 mL of DMEM containing 10% fetal bovine serum, supplemented with 50 U/mL penicillin and 50 µg/ml streptomycin and were incubated at 37 °C in 5% CO₂. All the treatments were done in triplicate for better understanding and reproducibility. After 24 h of culture, the medium in the wells was replaced with the fresh DMEM containing the samples in the concentrations of 50 µg/ml and were incubated for 24, 48 and 72 hr time interval. Then, MTT solution of 0.5 mg/ml in DMEM was added in each well and was incubated for another 3 hrs at 37°C to produce water-insoluble formazan. Finally, DMSO was added to each well to solubilize this formazan and absorbance was taken using microplate reader at 570 nm wavelength. The percentage cell viability was calculated using the formula.

$$\% \text{ Cell viability} = \frac{OD \text{ of Test}}{OD \text{ of Control}}$$

where, OD is the optical density of the specimen.

2.6 Fluorescence imaging: Proliferation of cell efficiency using different materials was visualized using fluorescence microscope. SiHa cells were seeded in 6-well plates at a confluency of 70-80 % on to the specimens for 24, 48 and 72 hr at 37°C. Test samples were washed twice with fresh PBS to remove the dead or floating cells. Adhered cells were fixed with 4% paraformaldehyde

solution for 20 min, washed with PBS again, and the samples were stained by using fluorescent dye acridine orange and ethidium dibromide (100 µg/ml) for 10 min followed by washing with PBS twice and were subsequently incubated for another 5 min in dark. Images were taken using fluorescence microscope (Leica, Germany).

Fluorescence quantum yield (ϕ_F) measurement in solution: The fluorescence quantum yield (ϕ_F) of TCPN1 and TCPN2 in tetrahydrofuran was measured relative to coumarin 314 in ethanol ($\phi_F = 0.68$)^{S1}. The quantum yield was calculated using the following equation.^{S2}

$$\phi_{F,x} = \phi_{F,s} \cdot \frac{F_x}{F_s} \cdot \frac{f_s}{f_x} \cdot \frac{n_x^2}{n_s^2}$$

Where, subscript “x” refers to unknown sample, and subscript “s” stands for standard sample. F denotes integral fluorescence, n indicates refractive index of the solvent used for measurement and f is the absorption factor at the excitation wavelength (380 nm),

$f = 1 - 10^{-\epsilon(\lambda_{ex}) \cdot c \cdot l} = 1 - 10^{-A(\lambda_{ex})}$, where A is the absorbance and ϵ is the molar extinction coefficient in L mol⁻¹ cm⁻¹.

Synthesis of TCPN1 and TCPN2: Similar experimental conditions were used to prepare both TCPN through the Suzuki-Miyaura coupling reaction between 3-substituted, 2,5 dibromo thiophene and ((1,3,5-Triazine-2,4,6-triyl)tris- (benzene-4,1-diyl))triboronic acid (TBBA). The following describes the synthetic procedure for TCPN1. TBBA (400 mg, 0.88 mmol), 2, 5-dibromo-3-hexyl thiophene (592 mg, 1.8 mmol) and K₂CO₃ (1.50 gm, 10.8 mmol) were put together in a round-bottomed flask with a solvent mixture of toluene, ethanol, and water (3:1:1). The catalyst Pd(PPh₃)₄ (314 mg, 0.272 mmol) was then added as needed, and the solution was immediately degassed using a vacuum-argon cycle for several times. The reaction mixture was then refluxed for 24 hr in an argon environment. After the reaction was finished, the product was

extracted by DCM by adding excess water. Solid product was recovered by evaporating the DCM solution. The product was further purified by re-dissolving in chloroform and precipitated in methanol. This dissolution in chloroform and precipitation in methanol was performed for several times to remove the impurities. NMR was used to confirm the structure of the polymer formed.

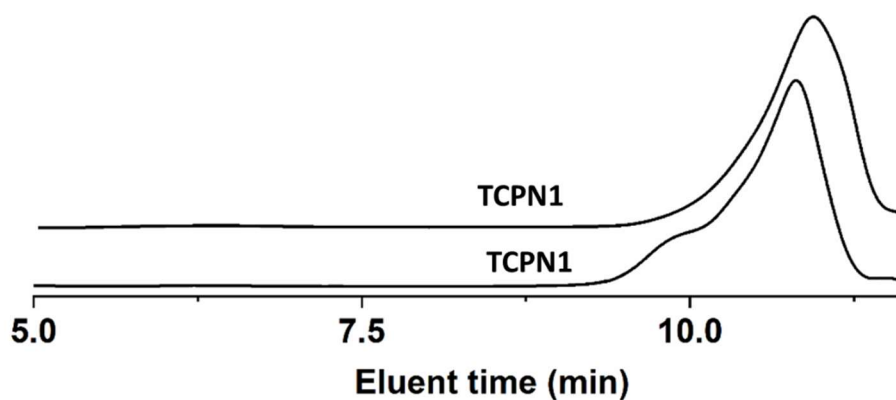


Figure S1 GPC traces of both TCPN using THF as eluent at 35°C.

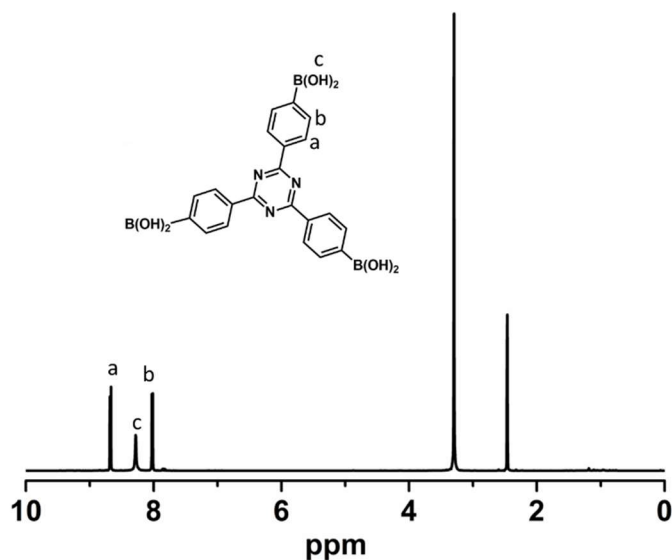
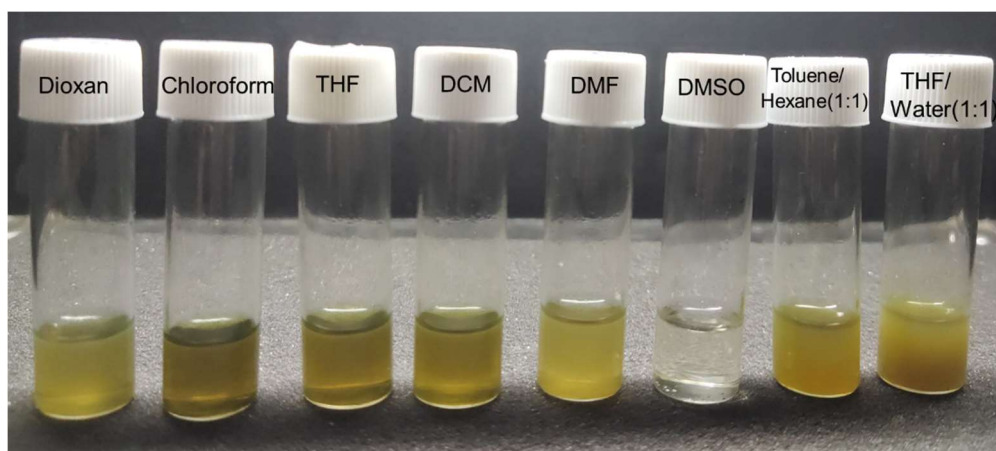
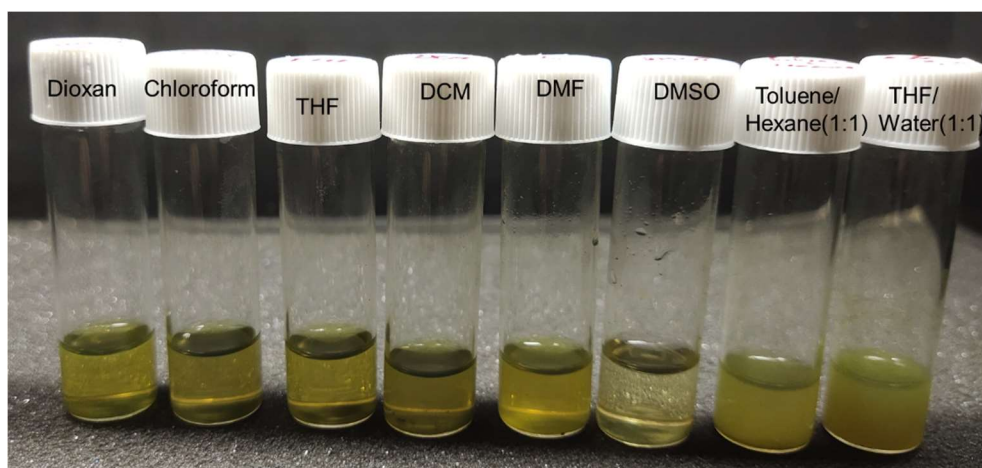


Figure S2 ^1H NMR spectra of ((1,3,5-triazine-2,4,6-triyl) tris (benzene-4,1-diyl)) triboronic acid (in d_6 DMSO).



(a)



(b)

Figure S3 Pictures of solution or dispersion in different solvent or solvent mixture of TCPN (1mg/ml), (a) TCPN1 and (b) TCPN2

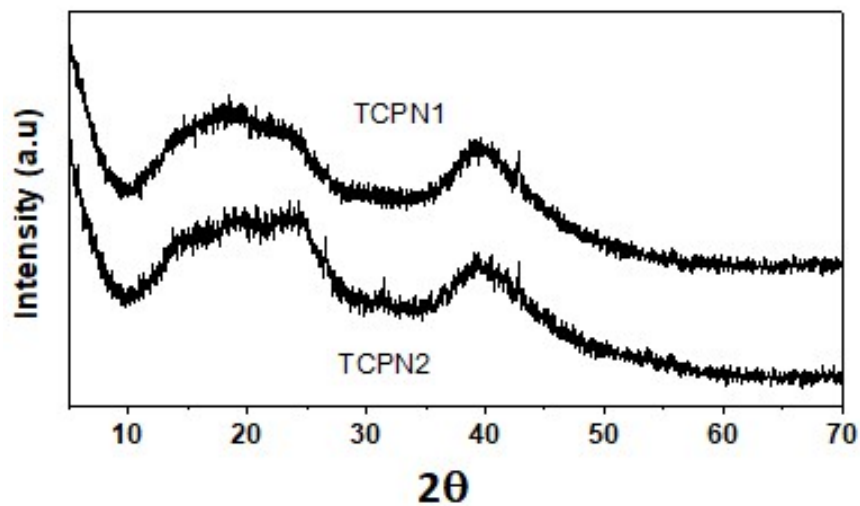


Figure S4 XRD pattern of TCPN1 and TCPN2

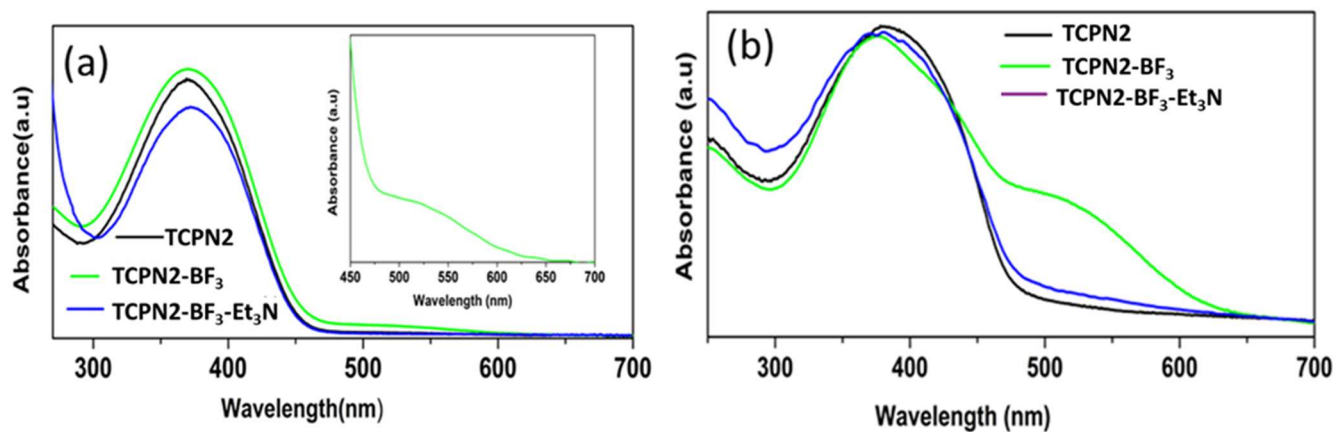


Figure S5 (a) and (b) UV-Vis spectra of pristine TCPN2, BF_3 doped and triethyl amine (Et_3N) dedoped in CHCl_3 and in thin film respectively, inset of figure (a) corresponds to enlarged portion between wavelength range 400 -700 nm.

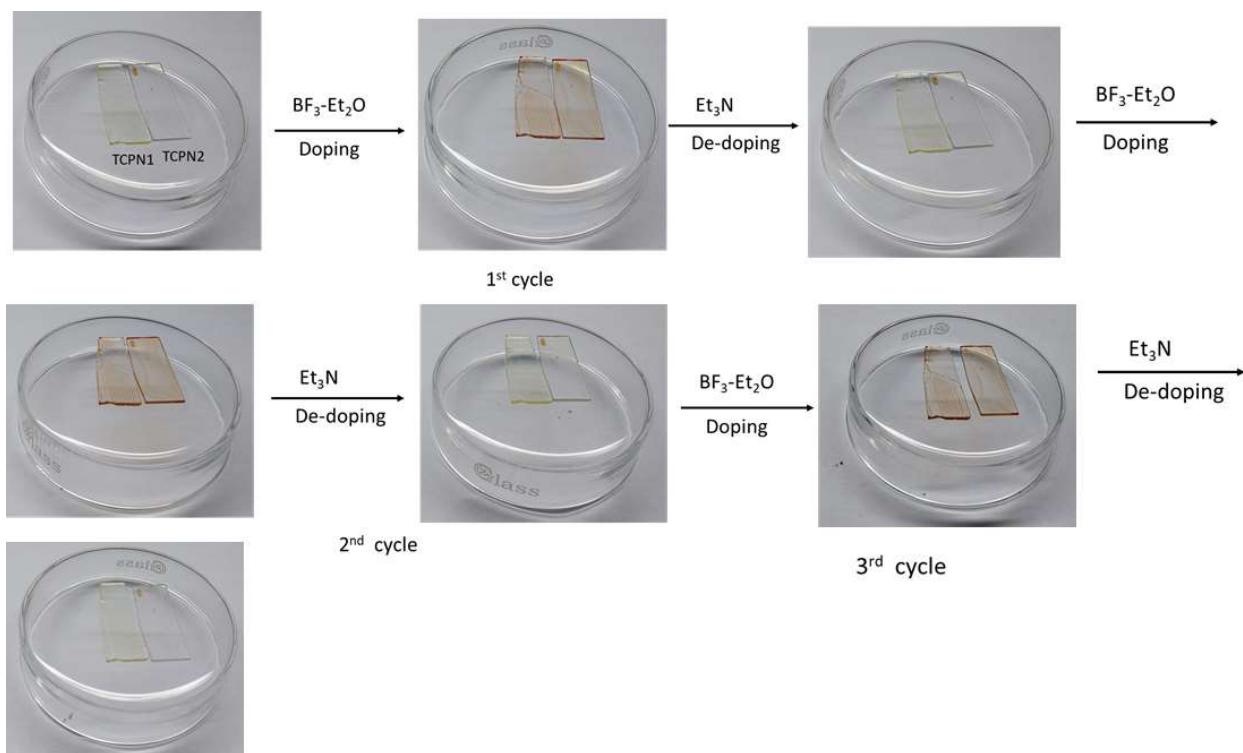


Figure S6 picture of the thin film of TCPN1 and TCPN2 after doping (exposing in $\text{BF}_3 \cdot \text{Et}_2\text{O}$ vapor for 10 minutes) and dedoping (exposing in Et_3N vapor 2 minute) for consecutive three cycles.

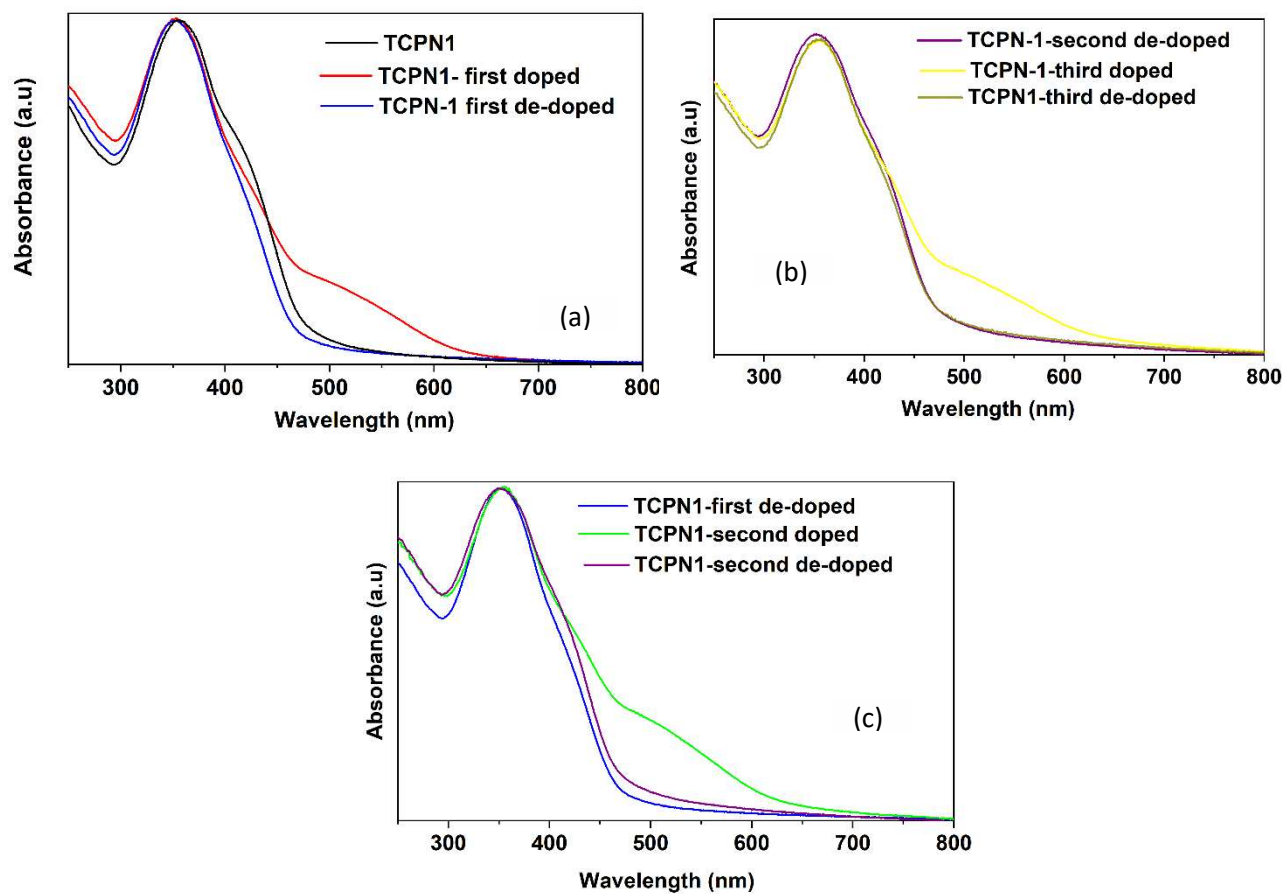


Figure S7 UV-Vis spectra of thin film of TCPN1 after doping (exposing in $\text{BF}_3\cdot\text{Et}_2\text{O}$ vapor for 10 minutes) and dedoping (exposing in Et_3N vapor 2 minute) for consecutive three cycles. (a) cycle 1 (b) cycle 2 (c) cycle 3

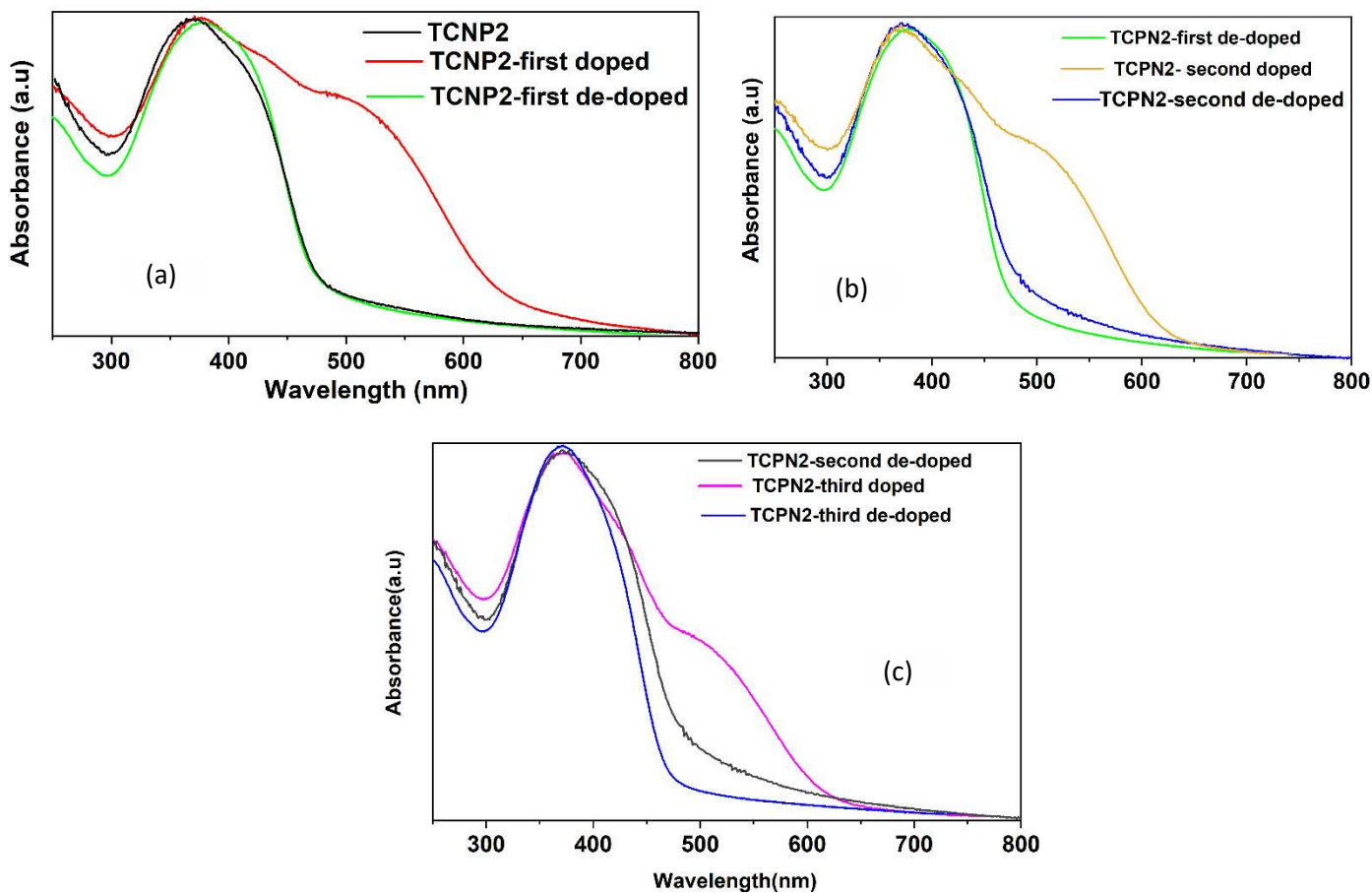


Figure S8 UV-Vis spectra of thin film of TCPN2 after doping (exposing in $\text{BF}_3\cdot\text{Et}_2\text{O}$ vapor for 10 minutes) and dedoping (exposing in Et_3N vapor 2 minute) for consecutive three cycles. (a) cycle 1 (b) cycle 2 (c) cycle 3

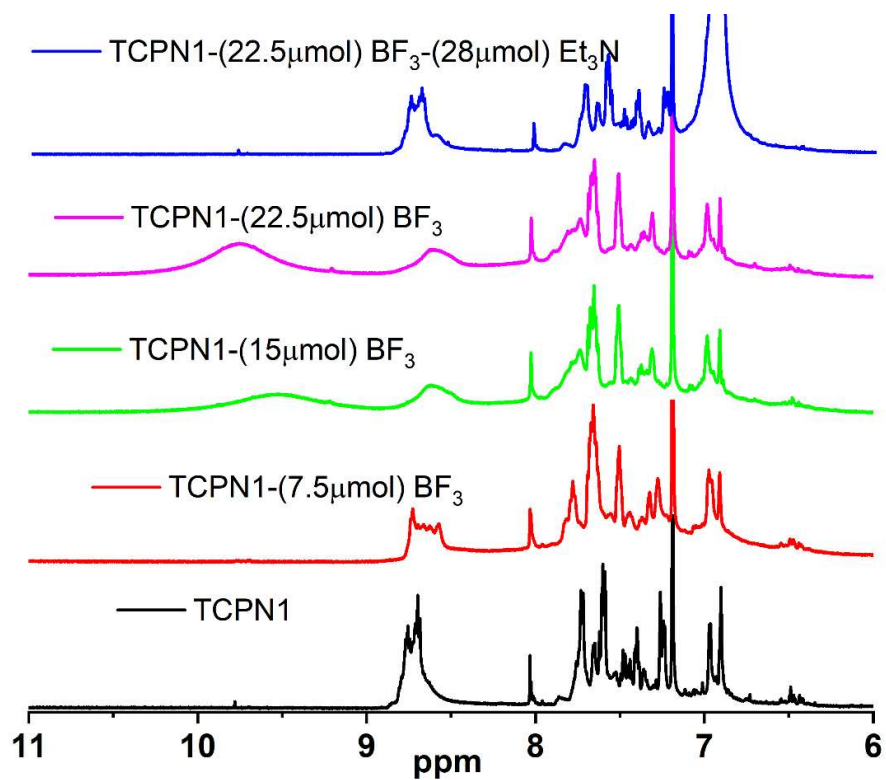


Figure S9 ^1H NMR spectra of TCPN1 in CDCl_3 after successive addition of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ and final addition of Et_3N .

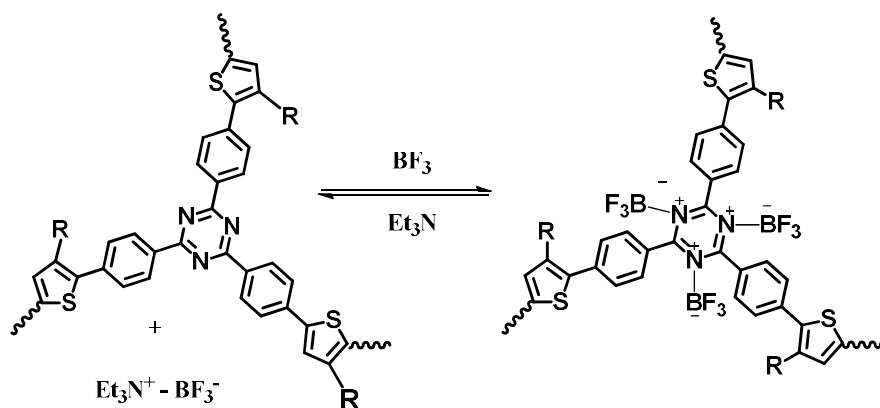


Figure S10 Proposed mechanism and doped structure formed by reaction of TCPN with Lewis acid.

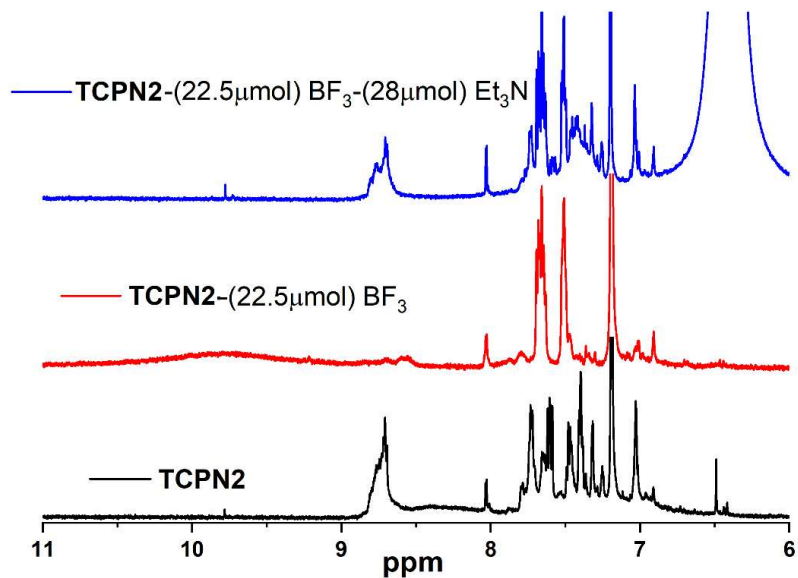


Figure S11 ^1H NMR spectra of TCPN2 in CDCl_3 after addition of $\text{BF}_3\cdot\text{Et}_2\text{O}$ and Et_3N .

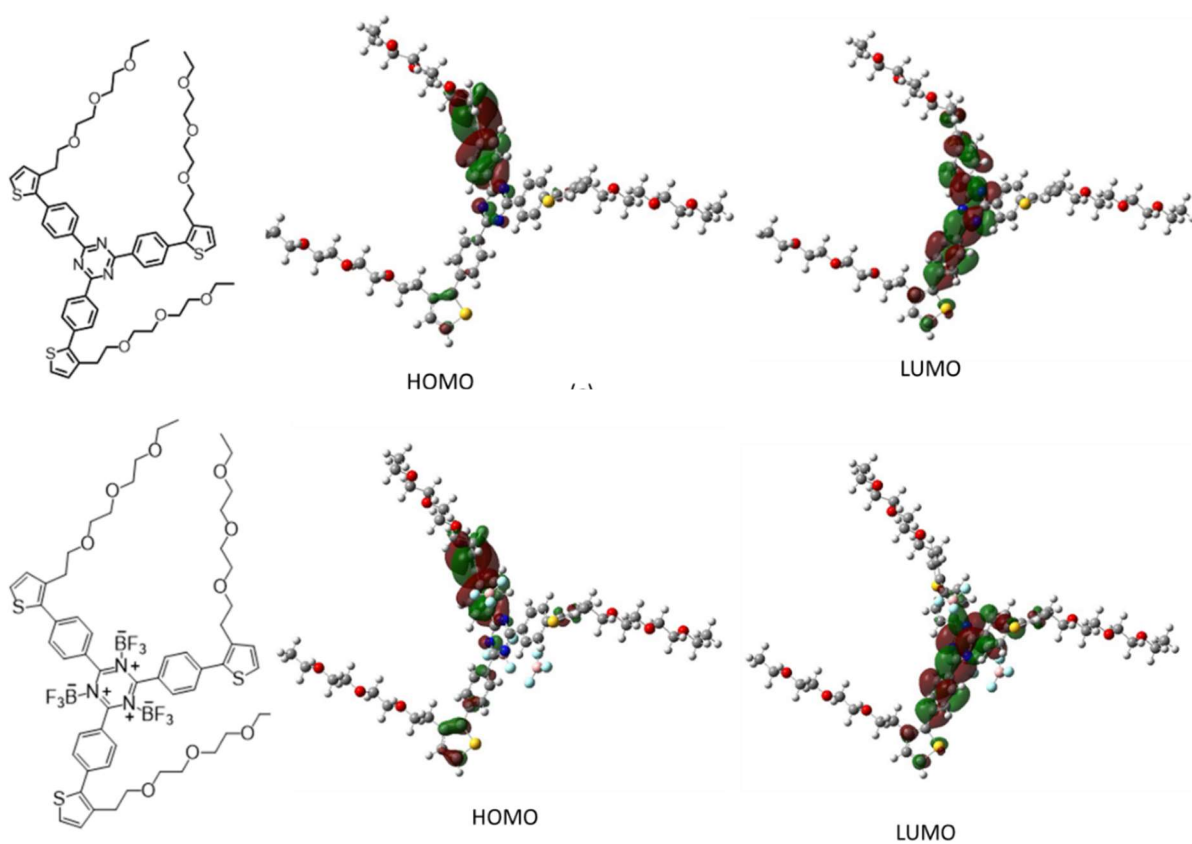


Figure S12 Chemical structure and topographical representations of HOMO and LUMO of TCPN2 (top) and BF_3 -TCPN2 (bottom).

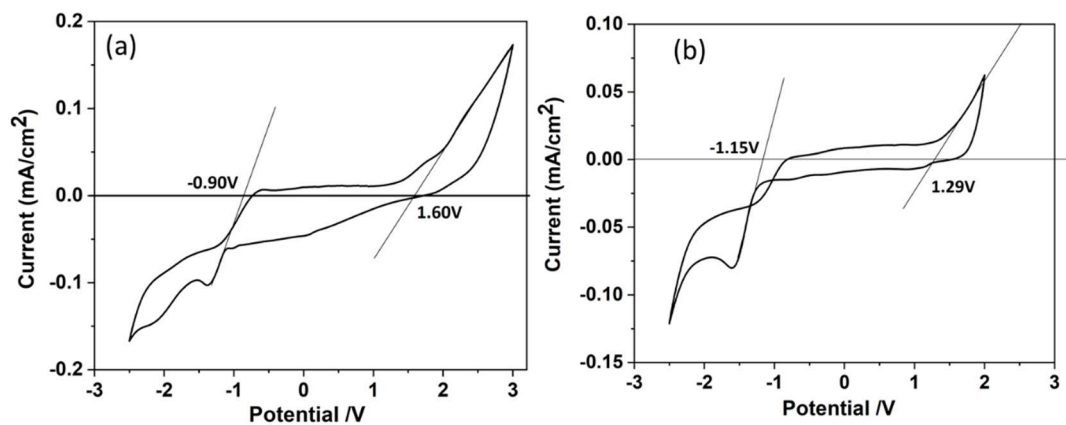


Figure S13 CV curve of TCPN for band gap calculation, (a) TCPN1 and (b) TCPN2. Cyclic voltammograms were measured in a CH_2Cl_2 solution of 0.1 M tetrabutylammonium hexafluorophosphate at 100 mV/s.

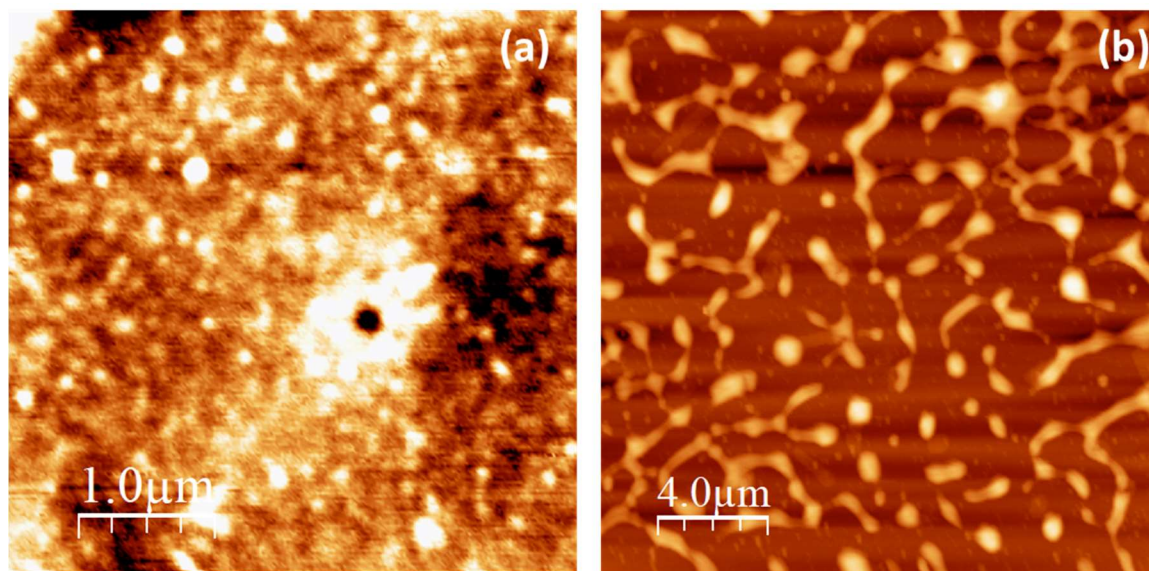


Figure S14 AFM height image of TCPN thin film deposited from THF (a) TCPN1 (b) TCPN2

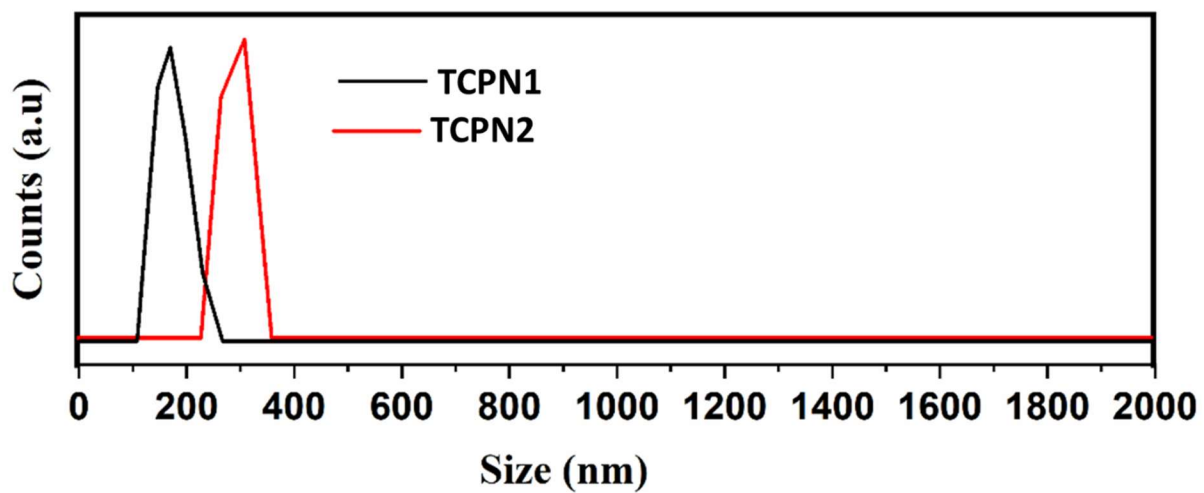


Figure S15 Dynamic light scattering plot of TCPN colloidal suspension in toluene/hexane mixture (1:1 by volume).

References:

S1 G. A. Reynolds, K. H. Drexhage, *Optics Commun.* 1975, **13**, 222-225.

S2 K. Rurak, M. Spieles, *Anal. Chem.* 2011, **83**, 1232.