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Supporting Information

Covalent Organic Framework membrane for Constant-Rate Peptide Release

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Experimental details:

1. Materials

Methylene blue (MB) and Triformylphloroglucinol (Tp) obtained from Toronto Research Chemicals (Canada) and Tokyo Chemical Industry Co., Ltd (Japan), repectively. Hydrofluoric acid (HF) was purchased from Acros Organics. 1,4-diaminobenzene dihydrochloride (DAB), PCL (MW 80K), poly(ethyleneglycol), oxytocin (OT), phosphate buffered saline (PBS), methanol, acetone, tetrahydrofuran (THF) and dichloromethane (DCM), Azobonzene (AB), and arcidine orange (AO) all were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chemicals were used as received without further purification. Wafers of diced silicon substrates (90 nm oxide, 20 mm x 15 mm) purchased from Ossila (UK) and were cut using dimond knife in the appropraite sizes and are used for the transferring of COF membranes. Ultrapure water (18 MΩ) was supplied by a Millipore System (Millipore Q, USA).

2. Synthesis of COF-LZU1 membrane

The imine-based COF (COF-LZU1) membrane was synthesized in the interface of two immiscible solvents by condensation of monomers. 2,4,6-Triformylphloroglucinol (Tp) (10.0 mg, 0.3 mmol) is dissolved in 30 mL dichloromethane (DCM), and 1,4-diaminobenzene dihydrochloride (DAB) (10.0 mg, 0.45 mmol) monomer is dissolved in water (30 mL). Firstly, dissolved Tp in dichloromethane was added to the home-made glass reactor (Figure S1), then the aqueous DAB is gently added to the top of monomeric solution of DCM while preventing bubble formation in the interface. After 7 hours, a continuous thin film in the interface is visible with a few hundred nanometer size thickness which can be transferred to the silica substrate placed in the bottom of reactor on the glassy ring by opening the valve located in the bottom of reactor. While the solvents are exiting the reactor, the membrane is being transferred on the silica substrate. This step should be done very slowly and cautiously to prevent the thin film to be broken. After drying the thin film onto the silica substrate for 4 h, water and ethanol were used to wash formed particles, and unreacted monomers for 10 min to gently rinse the COF thin film.

The post-treatment was conducted at 120 °C for 3 days in a teflon lined stainless steel autoclave filled with water/DCM (1:1, volume ratio). After cooling to room temperature, the crystalline COF thin films were washed with THF, MeOH, and acetone several times, and then dried at room temperature.

3. Testing and Characterization Methods

Fourier transform infrared (FT-IR) spectroscopy was recorded on Spectrum One (Perkin Elmer Instruments Co.Ltd.) with KBr as the background. Powder X-ray diffraction (PXRD) was performed using D/MAX-TTRIII (CBO) (Rigaku Corporation) with Cu Kα radiation (λ=1.54 Å) at a scanning rate of 6° min^{-1}. Transmission electron microscopy (TEM) images were carried out using Tecnai G2 F20 S-TWIN at 200 kV. The sample for TEM visualization is prepared as follows: the membranes on silica substrates are etched using (HF 20 % V) and both initial layers and thin layers of the membrane which is thinner than the initial layer are tansferred to the carbon coated copper grids. A field emission scanning electron microscope (SEM, Hitachi SU8220) was utilized to observe the surface and cross-section morphologies of COF membrane on silica substatre. Water contact angle measurements were performed with an Easy-Drop Instrument (JC2000D) at room temperature using the drop method. All membranes were dried at 40 °C prior to measurement.

4. Fabrication of the drug release device

4.1. Membrane transfer to porous PCL substrate

The membrane transfer process from the silica substrate to a porous PCL layer by etching method is shown in Figure S2. Firstly, PCL (Mn=80,000) is dissolved in dichloromethane (DCM) (500 mg/ml) and well mixed with poly(ethyleneglycol) (PEG; Mn= 2,000) (150 mg/ml) in DCM on the magnetic stirrer until well dissolved. A model of KW-4A series SPI spin coater (West Chester, PA) is used for casting thin films, firstly at 1500 rpm for 30 seconds followed by spinning at 2000 rpm for 60 seconds.

We have casted PCL films on two different substrates; silicon wafer (see Figure S2, 1st step) and COF membranes on silicon wafers with ultrathin silicon oxide coating (see Figure S2, 3nd step). PCL/PEG solutions in DCM were spin casted onto clean silicon wafers. In order to dissolve PEG from PCL film and detach PCL film from silicon wafer, the resulting thin films were incubated at 37 °C for at least one hour and half to let films separated from wafer silicon. In the step for making PCL/COF layer, solution of polymers is spin coated onto the surface of COF thin film one silica substrate (with ultrathin silica oxide layer), then this thin film composite of PCL/PEG and COF is being etched from the silica using (HF 20 % V) as it is depicted in Figure S2, 2nd step, the floated membrane then was transferred to pre-synthesized PCL thin film which its PEG part was dissolved previously. The sandwich structure of PCL/COF/PCL (about 1 cm^2) were punched out using micro-punches (CaronnoPortusella, Milan, Italy), and placed on the top of printed drug delivery device (Figure S2, 4th step).

4.2. 3D printing of PCL drug release device

A three-dimensional drug delivery container was created in Creo Parametric computer aided design software as shown in Figure 1a. 3D printing was conducted using an Ultimaker 3D printers at temperatures ranging from 120 °C to 125 °C (dimensions; Ø: 25 mm, t: 10 mm).

4.3. Assembly of device

The devices herein were created with three elements: a nanoporous sandwiched layers of PCL/COF/PCL, peptide (10 mg), and nonporous 3D printed PCL container that encloses that peptide. Devices were sealed utilizing a hot plate as a uniform heat source, and a stacked apparatus was used to assemble the device layers for heat sealing. To provide structural support and allow conformal contact between the layers of the device and decreasing heating to the peptide and membrane, an annulus and upper support were fabricated from poly-(dimethylsiloxane) (PDMS).

5. ***In vitro* release experiments of dyes and peptide**

Prior to all elution studies, devices were vortexed (40 seconds) in phosphate-buffered saline (PBS). To measure the speed of release, devices were placed in PBS at 37 °C to simulate physiological conditions and the contents of the reservoir were periodically exchanged. All the systems were kept slowly shaken in the dark room. Uv-vis spectroscopy was performed on elution samples (n = 3) and concentrations of eluted dyes were extrapolated based on a standard curve in the linear range (Figure S5). The amount of peptide in the elusion samples (n = 3) was determined by an OT-specific enzyme linked immunosorbent assay (ELISA) (Oxytocin ELISA kit, Catalogue no: ADI-901–153A-0001, Enzo life Sciences, NY US).

6. ***In vitro* studies**

6.1. Cell culture

NIH 3T3 fibroblast cell line was purchased from American Type Culture Collection, ATCC (VA, US) and was subcultured in High Glucose Dulbecco's modified Eagle's medium (HG-DMEM, Sigma-Aldrich, USA) supplemented with 10 % fetal bovine serum (FBS, Sigma-Aldrich, USA) and 1 % penicillin-streptomycin solution (P/S, Sigma-Aldrich, USA) under 99 % humidity and 5 % CO_2 at 37 °C. The cells were passaged following washing, trypsinization and splitting into various batches.

6.2. Optical observations of cells and CCK-8 assay

Cell viability were measured using Cell Count Kit-8 (CCK-8) (Dojindo Laboratories, Japan). The CCK-8 contains WST-8 [2-(2-methoxy-4-nitrophenyl)- 3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] which becomes a water-soluble formazan dye upon reduction with the dehydrogenase in the mitochondria. The samples were cut into sizes to suit the weight equivalent of 250 µg for PCL and COF thin films (250 µg, 125 µg, 100 µg, 50 µg, 25 µg), and pre-wetted by immersion in the culture medium for 1 h in a 37 °C incubator. Briefly, 100 µl of cell suspension (6000 cells/well) dispensed in a 96-well plate. Then, the plate is incubated for an appropriate length of time (24, and 48 hours) in a humidified incubator (e.g., at 37 °C, 5 % CO_2). The cell morphology were observed under optical microscopy (Zeiss, Axio observer). After treatment, the culture medium was discarded and 100 µL of fresh culture medium containing 10 % CCK-8 was added to each well. After incubated for 2 h at 37 °C, the value of the absorbance (A) at 450 nm was measured by using

a microplate reader (TECAN, Durham, USA). The cell viability (%η) was calculated by the equal (1):

$$\%\eta=(A_{sample}-A_{blank})/(A_{control}-A_{blank})\times 100\% \quad (1)$$

6.3. Cell morphology of the samples

For experiments of cell proliferation and adhesion, samples (about 1 cm^2) were punched out using micro-punches (CaronnoPortusella, Milan, Italy), and placed on the bottom of a sterile standard 48-well plate. For cell proliferation study, NIH 3T3 cells were respectively seeded onto each sample at a density of 0.03 x 10^6 cells for 24 h, and 48 h. After the incubation time, the seeded samples were rinsed with PBS and fixed with 2.0 % paraformaldehyde and 2.5 % glutaraldehyde (both E.M. grade) in 0.1 M Na-Cacodylate buffer, pH 7.4) at 4 °C for 12 h. For assessment of morphology, the fixed samples were subjected to a drying process by passing them through a series of graded alcohol–PBS solutions (30, 50, 70, 80, 90, 95 and 100 %, 15 minutes each) and hexamethyldisilazane (HDMS)–alcohol solutions (30, 50, 70, 80, 90, 95 and 100 %, 15 min each in a fume hood). The specimens were sputter-coated with an Au/Pd layer and observed by a SEM.

6.4. Confocal microscopy study

NIH 3T3 cells were seeded at the density of 1 × 10^5 per dish in special glass dishes and allowed to attach for 24 h. Then, COF suspensions in DMEM at a final concentration of 250 μg/mL were added in. After 24 h of incubation, the medium was removed and the cells were washed with cold PBS (pH 7.4) three times. Trypan blue PBS solution (0.4 %) was added to quench any fluorescence outside the cells for 10 min. Afterwards, the cells were fixed with 4% paraformaldehyde at room temperature for 10 min and extracted with 0.1 % Triton X-100 in PBS for 3 min. Subsequently, the filamentous actin cytoskeleton was stained with 200 ng/mL rhodamine phalloidin for 20 min, followed the nuclei staining with DAPI for 10 min. Finally, the samples were analyzed with the laser scanning confocal microscope (LSCM, Zessi LSM 710, Germany).

7. Statistical Analysis

Statistics were performed with GraphPad Prism 5.0 (GraphPad, USA) using one- or two-way ANOVA tests with Tukey's multiple comparisons correction. Statistical difference between

two sets of data were analyzed by a Student's t test. Values are presented as mean ± standard deviation (SD). Differences were considered statistically significant for $p < 0.05$.

Supplementary Figures:

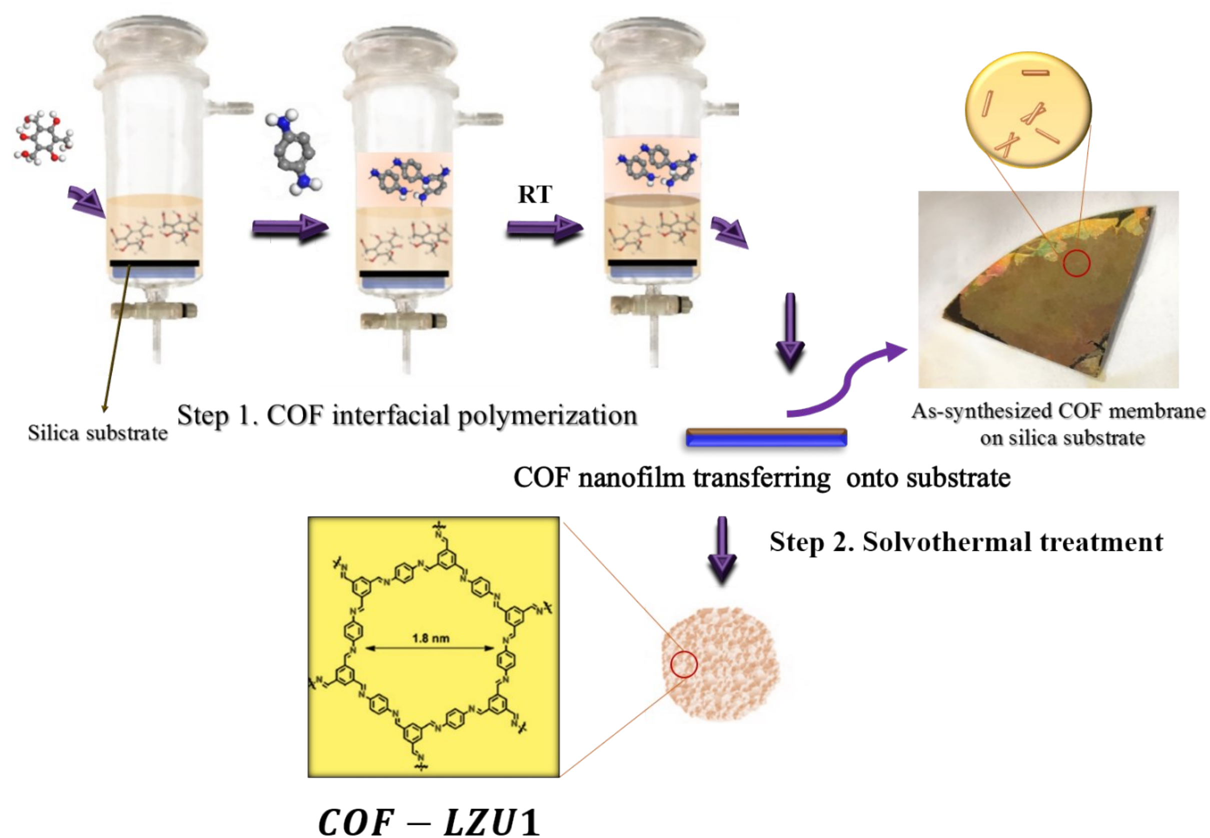

Figure S1. Schematic illustration of the preparation of COF-LZU1 membrane by interfacial polymerization, and the subsequent thermal treatment to improve the crystallinity.

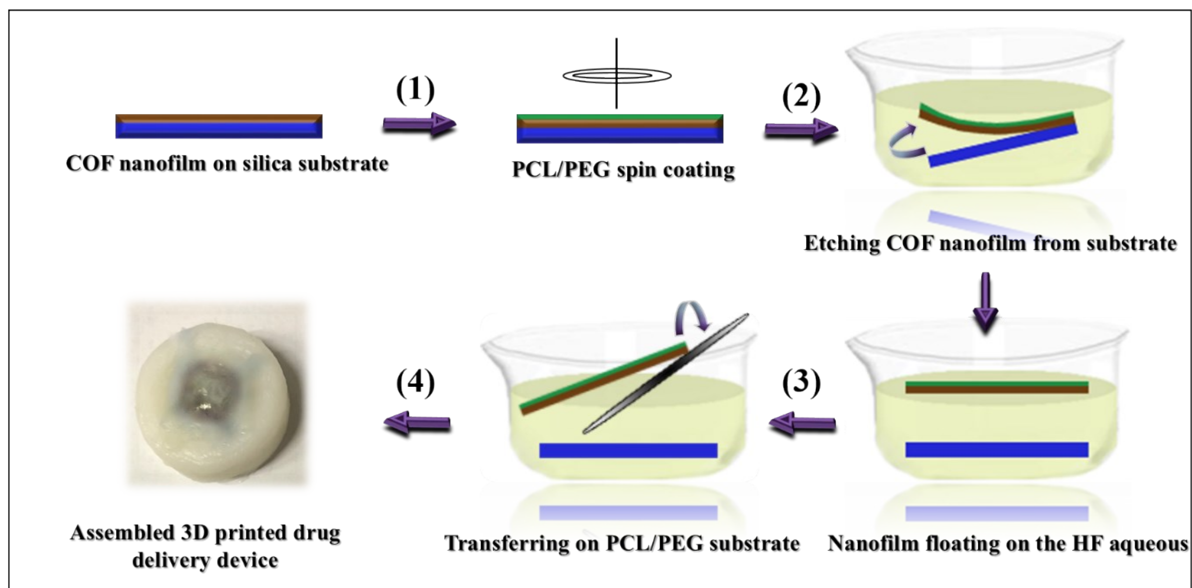

Figure S2. Membrane transfer to PCL/PEG substrate (1-3), and the assembly of drug release device (4).

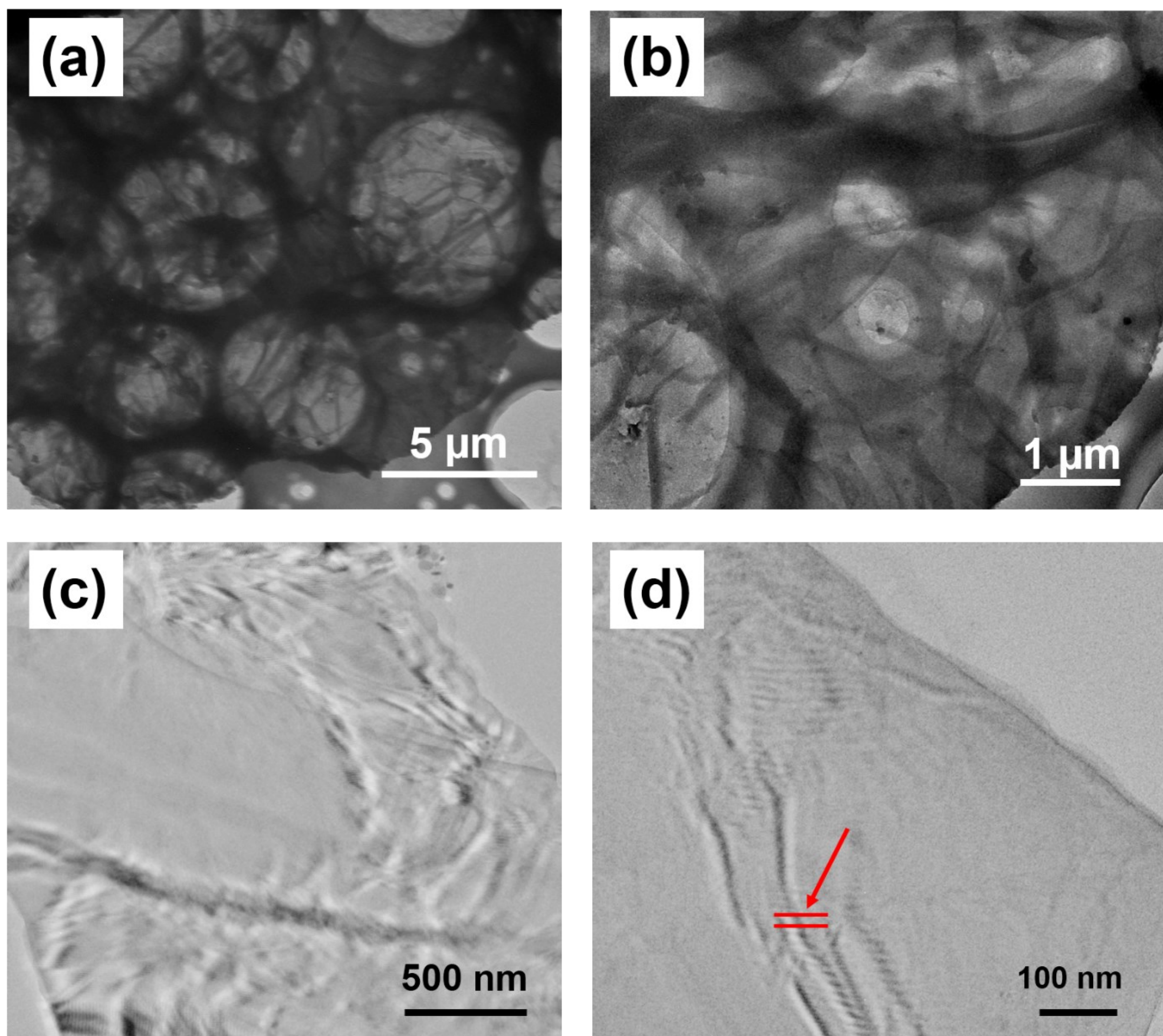

Figure S3. a,b) TEM images of monolith COF-LZU1 membrane in different magnifications. c, d) TEM images of membranes with thinner thickness, showing the moire fringe caused by the stacking of two crystalline layers as shown with red arrow in d.

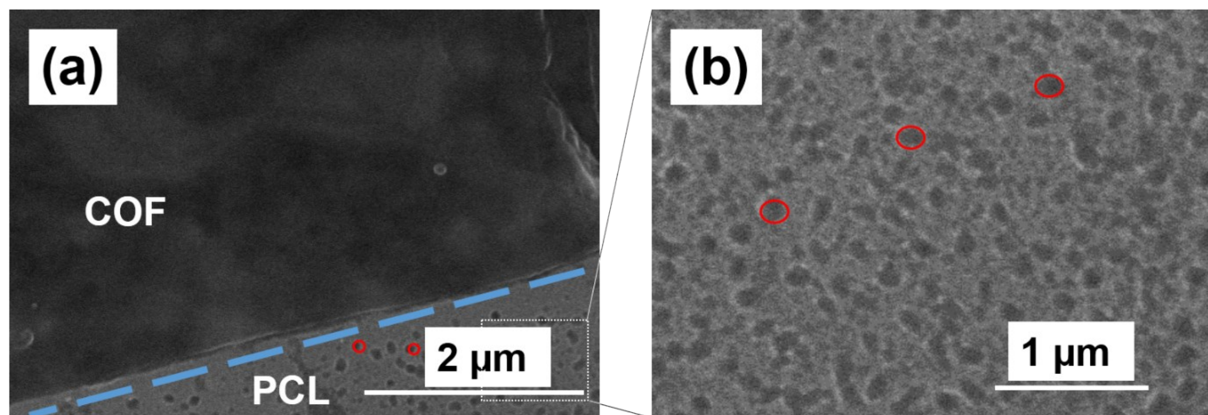

Figure S4. a) SEM image of porous PCL membrane with pore size of 193 ± 44 nm. b) SEM images of COF membrane on PCL substrate. Red circles show the typical pores of the PCL membrane.

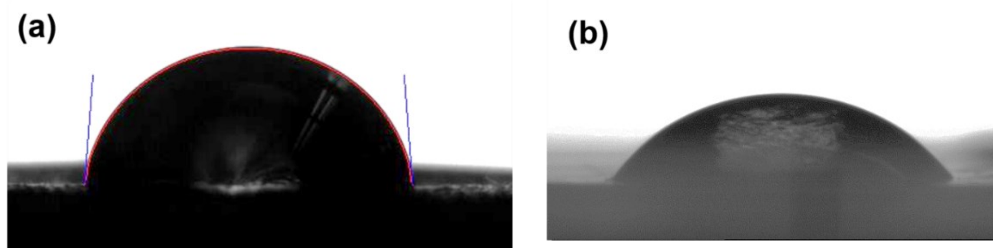

Figure S5. Water contact angle measurements of (a) PCL and (b) COF membrane, with values of 86º and 52º, respectively.

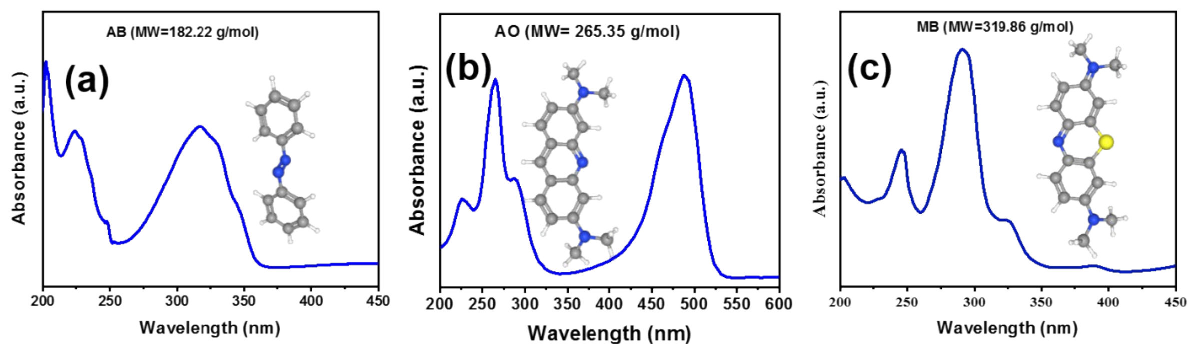

Figure S6. UV-Vis spectrum and chemical structures of dyes with different molecular weight (MW) used in this study. The molecular weight of dyes is 320 g/mol for Methylene Blue (MB), 265 g/mol for Arcidine Orange (AO), and 188 g/mol for Azobenzene (AB). (gray for carbon, white for oxygen, blue for nitrogen, and yellow for sulfur). The excitation peaks at 319 nm, 488 nm, and 290 nm are used for the concentration measurements of AB, AO, and MB, respectively.

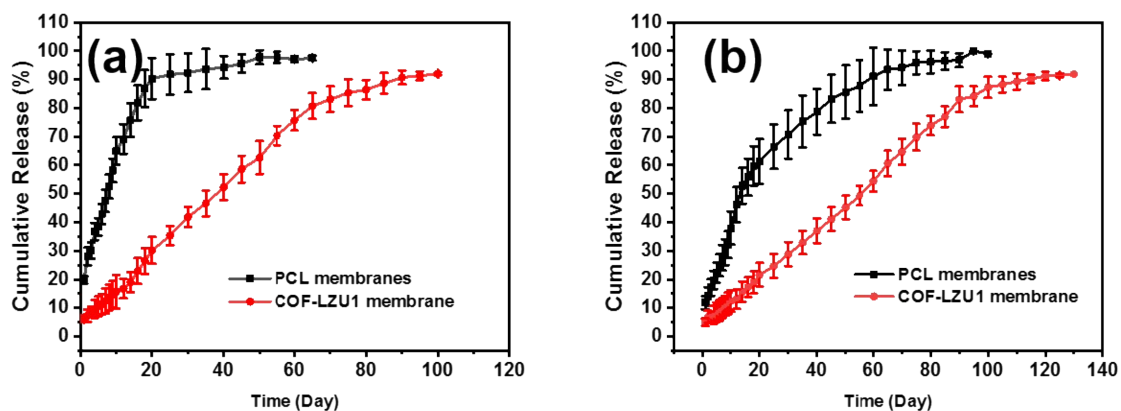

Figure S7. The release patterns of AB (a), and AO (b) through PCL membranes (black) and PCL/COF/PCL membranes (red).

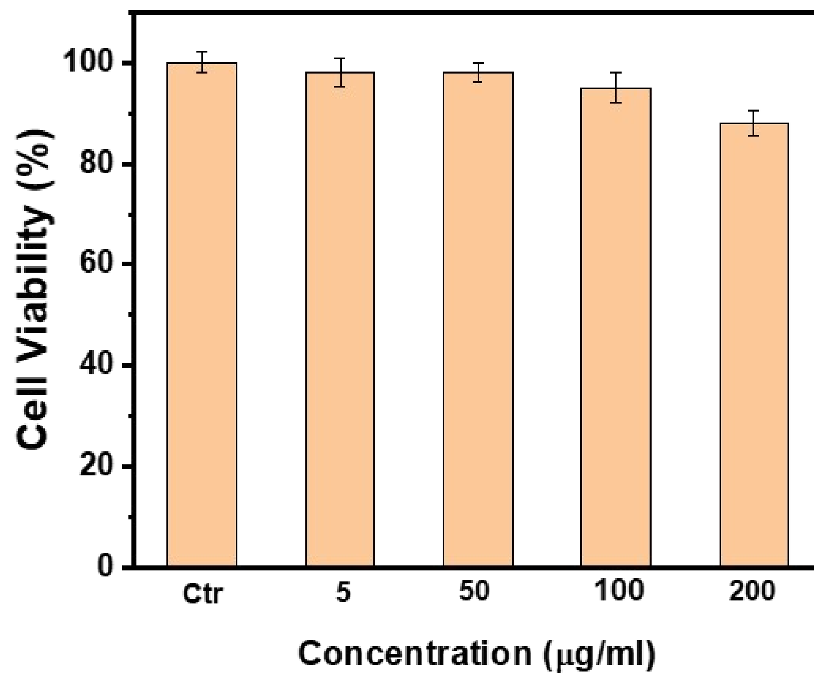

Figure S8. Cell viability evaluation of COF materials. The COF membrane was incubated with NIH 3T3 cells with different concentrations for 24 h. Control (Ctr) are the cells which are not incubated with COF materials.

Supplementary Tables:

Table S1. Molecular weight, charge (at pH=7), and size of tested dyes and peptide in this study measured through Material Studio.

Name	charge	Molecular weight (g/mol)	size
Azobenzene (AB)	+	188.22	1.1 nm*0.431 nm
Arcidine Orange (AO)	+	265.35	1.28 nm*0.42 nm
Methylene Blue (MB)	+	319.86	1.25 nm*0.51 nm
Oxytocin (OT)	neutral	1008	1.8 nm*0.9 nm

Table S2. Parameters of zero-order release kinetics of small dyes through COF-LZU1 membrane.

COF-LZU1	K_o (zero-order constant)		*Time$_{60\%}$ (day)	
	Only PCL membranes	PCL/COF/PCL membranes	Only PCL membranes	PCL/COF/PCL membranes
Azobenzene (AB)	-	0.98±0.007	10±2	45±2
Arcidine Orange (AO)	-	0.826±0.04	15±4	65±3
Methylene Blue (MB)	-	0.79±0.05	25±3	70±2

*Time$_{60\%}$: Time has passed from 0% cumulative release of molecules to 60 % cumulative release.