Supporting Information

Graphene Oxide Functionalized Membranes: The Importance of Nanosheet Surface Exposure for Biofouling Resistance

Authors

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S1. Dehydration of Cells for SEM Characterization

Karnovsky solution: Paraformaldehyde (8 g) was dissolved in 100 mL DI water at 60 °C, and NaOH solution (1 M) was slowly added until the solution became clear. The solution was then filtered using a 0.22 μm PVDF membrane and mixed with glutaraldehyde (20 mL) and cacodylate buffer (80 mL). After thorough mixing, the pH of the solution was adjusted to 7.4 by adding hydrochloric acid (1 M). The prepared solution was stored at 4 °C until use.

Dehydration procedure*:* After contacting the *E. coli* suspension (10^8 CFU mL^{-1}) for 3 hours, the membranes were gently washed with sterile saline solution (0.9 % NaCl) to remove the unattached cells from membrane surfaces. Then, the membranes were immersed in Karnovsky solution for 3 hours, and sequentially immersed in water/ethanol (50:50, 30:70, 20:80, 10:90, and 0:100) and ethanol/freon (50:50, 25:75, and 0:100) solutions for 10 min. After dehydration, the membranes were dried overnight in a desiccator at room temperature. Before SEM characterization, the samples were coated with a 10-nm thick layer of iridium.

S2. Confocal Laser Scanning Microscopy Analysis

The biofilm thickness and biovolume ratios that developed on the membrane surfaces at the end of the 8-hour dynamic biofouling experiment were characterized using confocal laser scanning microscopy (CLSM, Zeiss LSM 510, Carl Zeiss Inc.), using the protocol reported in previous studies.[1-3] After the 8-hour biofouling, the membrane coupons were taken out from the cross-flow UF cell and washed gently using the synthetic feed solution without *E. coli* (detailed composition is given in Table S1). The samples for CLSM measurement were cut from the center sections of membrane coupons and stained for 45 minutes in the dark with SYTO 9, propidium iodide (PI) (LIVE/DEAD BacLight, Invitrogen), and concavalin A (Con A, Alexa Flour 633, Invitrogen). SYTO 9, PI, and Con A were excited with 488 nm argon, 561 nm diode-pumped solid state, and 633 nm helium−neon lasers, respectively. CLSM stacking images were analyzed using Zen (Black edition), Auto-PHLIP-ML, Image-J, and MATLAB software.

S3. Antibacterial Test in Suspension

Concentrated GO stock suspension (6.2 g L^{-1}) and dopamine powder were added to the Tris buffer (pH 8.5) simultaneously to obtain a GO and dopamine mixture with a final concentration of 200 mg L^{-1} and 2 g L^{-1}, respectively. The mixture was allowed to react for 30 minutes. Then, the PDA/GO mixture was collected via centrifugation and washed with sterile saline solution three times to remove the unreacted dopamine monomer, unbound GO nanosheets, and replace the solvent from Tris buffer to saline solution. Dopamine powder (2 g L^{-1}) was dissolved in Tris buffer (pH 8.5) and the obtained solution was shaken for 30 minutes to obtain the pure polydopamine (PDA) particles. The pure PDA was collected through the same procedures as described above. In the antibacterial test, *E. coli* suspension with a concentration of 10^8 CFU mL^{-1} was added to the sterile saline solution, pure GO (200 mg L^{-1} in saline solution), pure PDA, and PDA/GO mixtures. After shaking for 3 hours, the suspensions were serially diluted and incubated on the LB agar plates overnight at 37 °C for CFU enumeration. The particle size and zeta potential of the suspensions were measured by Dynamic Laser Scattering (DLS, NanoBrook, Brookhaven).

Figures

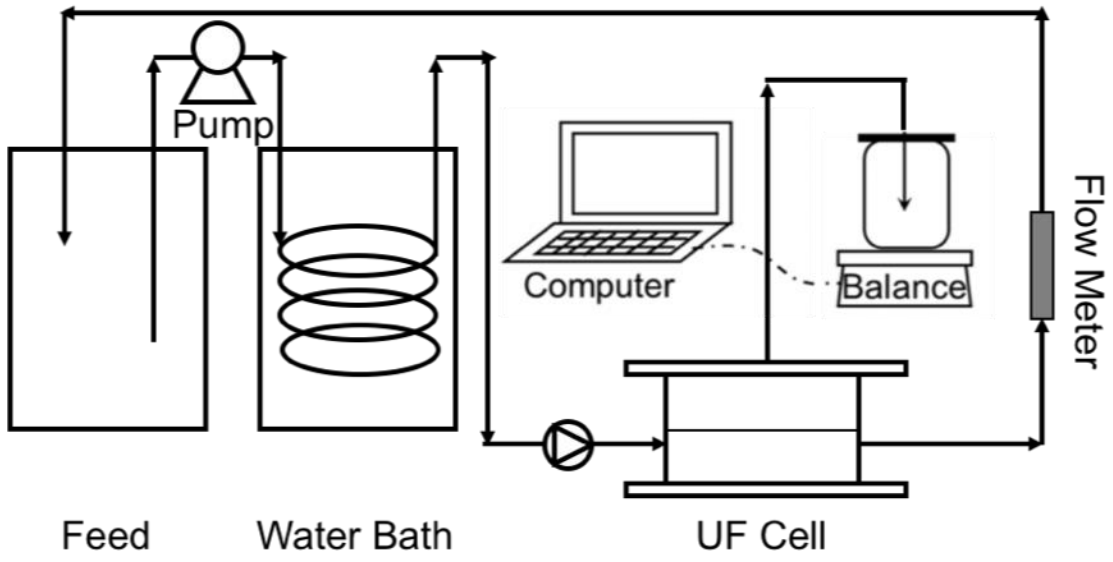

Figure S1. Schematic diagram of the cross-flow UF setup. The dimensions of the UF membrane cell are 25 mm in length, 25 mm in width, and 3 mm in height. The effective area of membrane is 625 mm^2. The cumulative permeate volume was recorded for flux calculation during the dynamic biofouling and biofouling reversibility experiments. For all the experiments, the crossflow velocity and the temperature were maintained at 11.1 cm s^{-1} and 25 ± 0.5 °C, respectively.

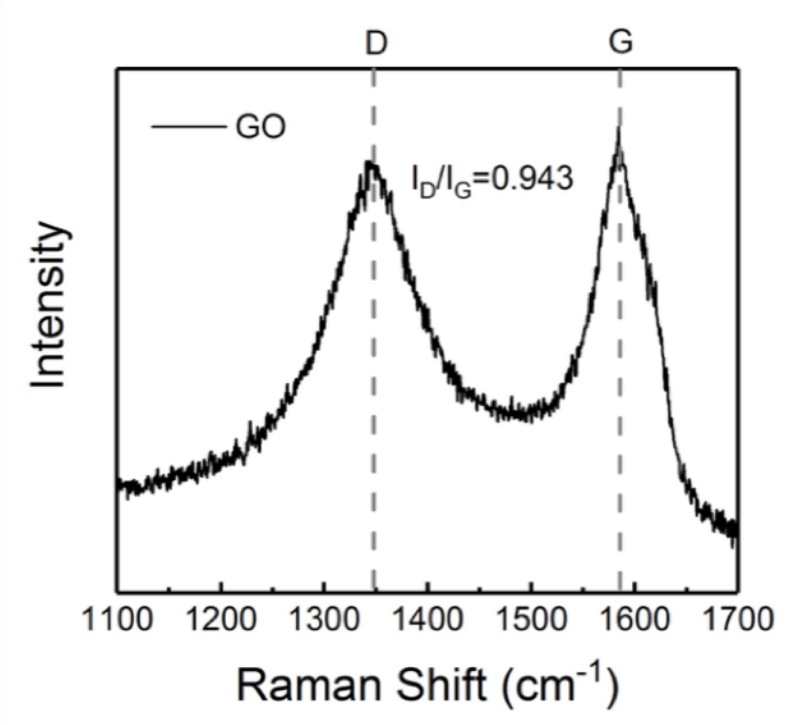

Figure S2. Raman spectrum of the pristine GO. GO suspension (6.2 g L^{-1}) was drop cast on a clean silicon wafer and evaporated in a fume hood to achieve dry GO sample for characterization.

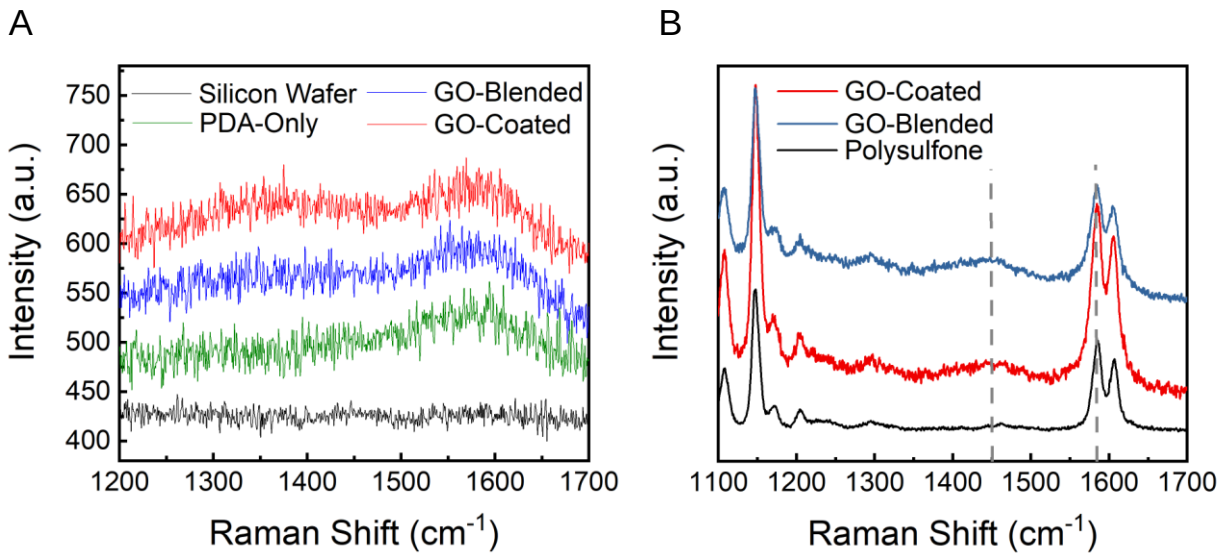

Figure S3. (A) Raw data of the Raman Spectra for PDA-only, GO-coated, and GO-blended silicon wafers. (B) Raman Spectra of the polysulfone, GO-coated, and GO-blended polysulfone membranes. A shifted band from 1350 cm^{-1} to 1450 cm^{-1} was also observed in a previous study on the GO modified membrane.[4]

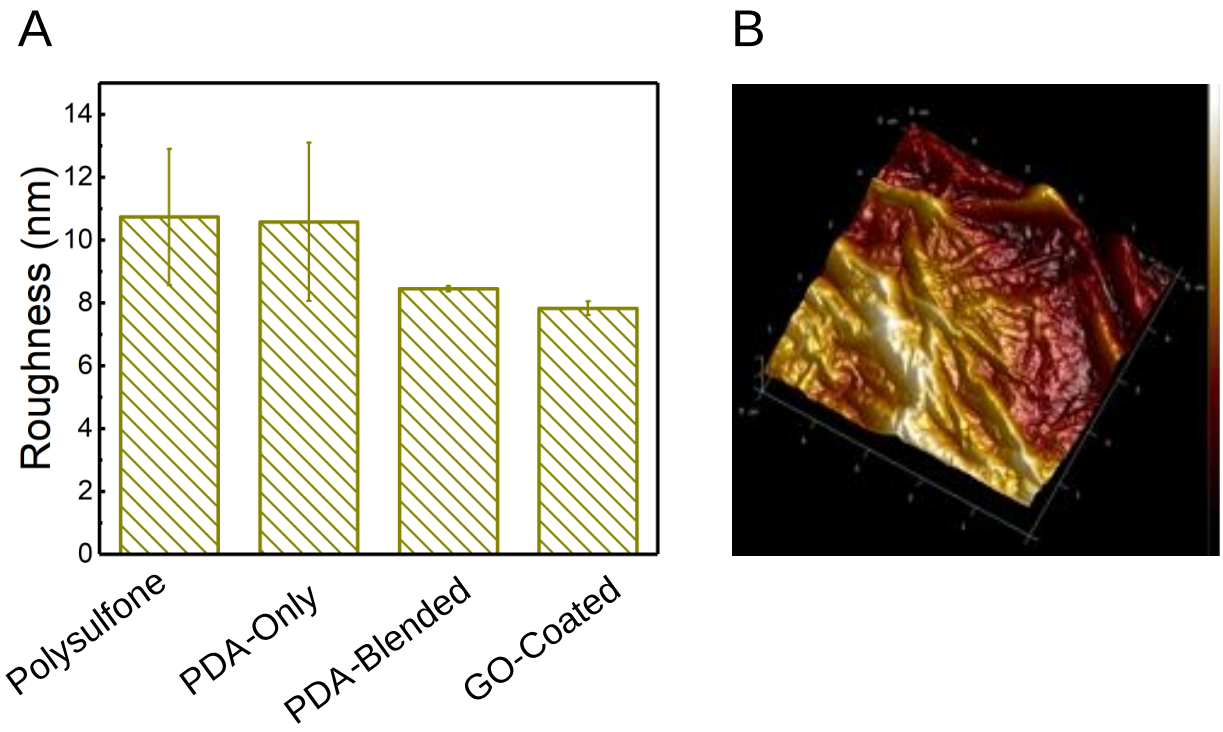

Figure S4. (A) Average roughness (R_a) of the polysulfone, PDA-only, GO-blended, and GO-coated membranes. (B) AFM images of the high-loading GO-modified membrane fabricated by the filtration method.

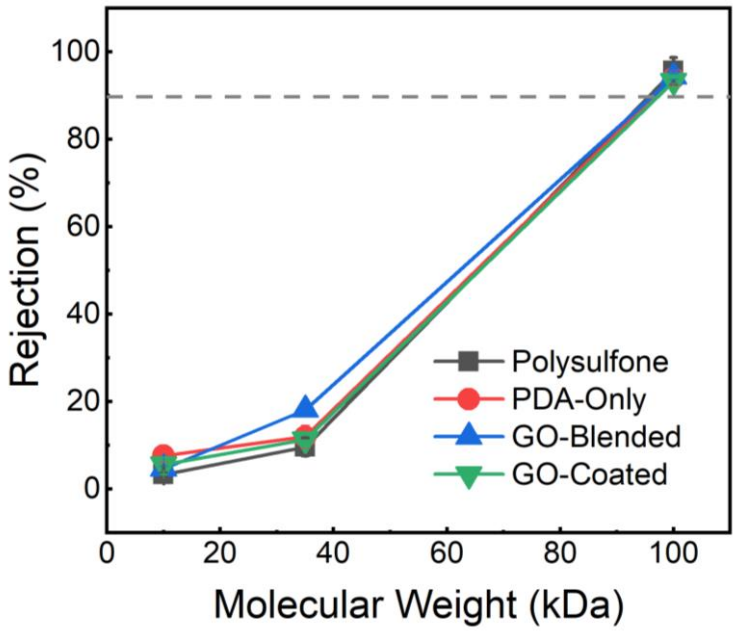

Figure S5. PEG rejection of polysulfone, PDA-only, GO-blended, and GO-coated membranes. PEGs with different molecular weights (i.e., 10, 35, and 100 kDa) were used to determine the molecular weight cut-off (MWCO) of the membranes. Experiments were carried out at an applied pressure of 2.5 bar. Rejection of PEGs was measured by a total organic carbon analyzer. The molecular weight of the PEG corresponding to 90% rejection in filtration experiments was defined as the MWCO of the membrane.

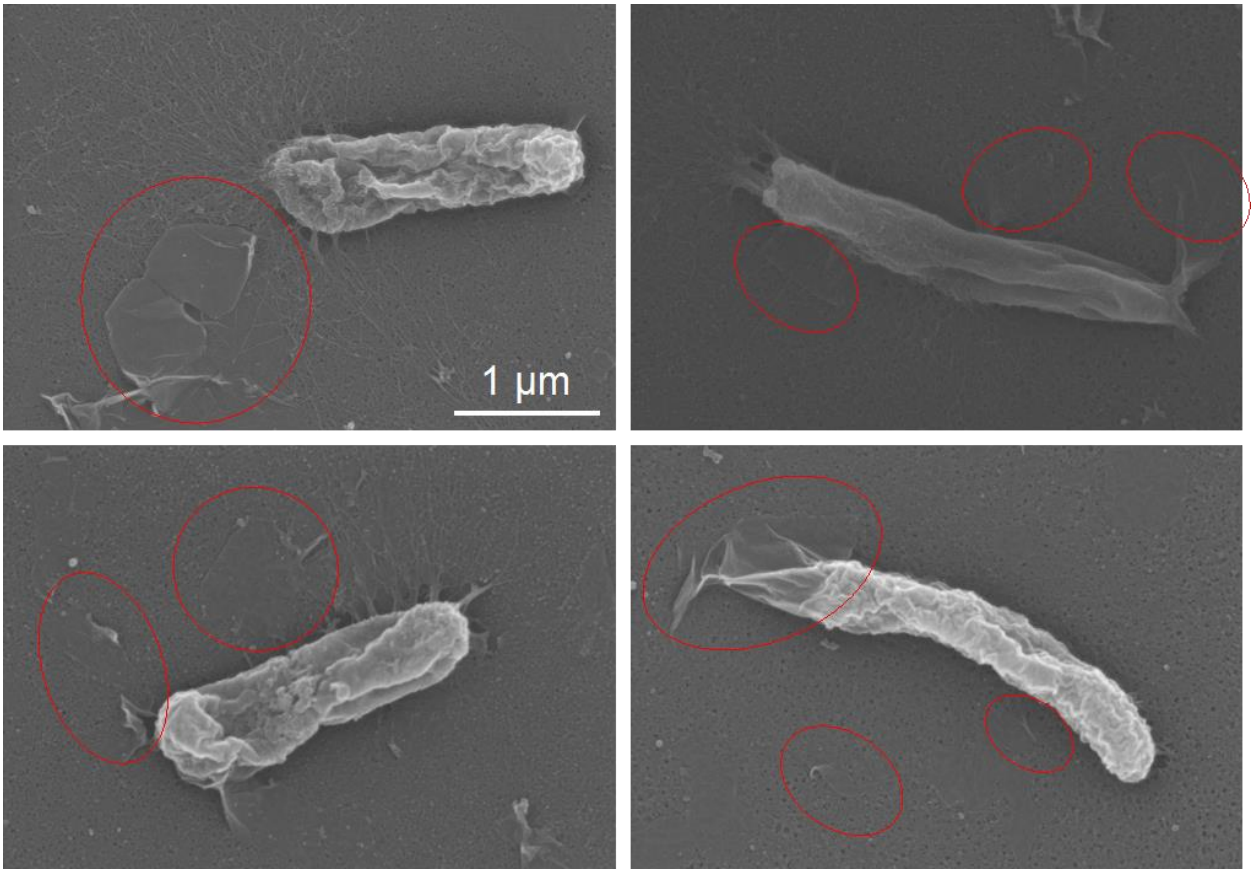

Figure S6. SEM images of the *E. coli* cells on the surface of GO-coated membranes after 3 hours static contact test with 10^8 CFU ml^{-1} of *E. coli* suspension. The samples were washed with sterile saline solution, dehydrated, dried, and sputter-coated with iridium as described above before SEM characterization. The red circles indicate the exposed GO nanosheets/edges on the membrane surfaces.

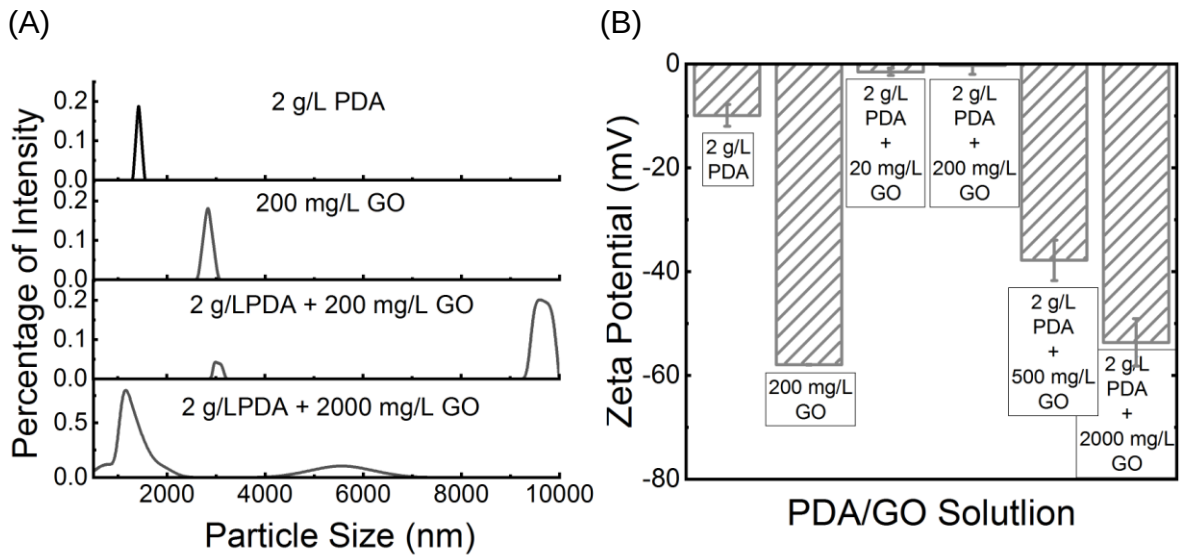

Figure S7. (A) Size distribution of the pure PDA, pure GO, and PDA/GO mixture via dynamic light scattering (DLS) measurement. (B) The average particle size and zeta potential of the PDA/GO suspensions. Error bars represent ± s.d. for five independent measurements.

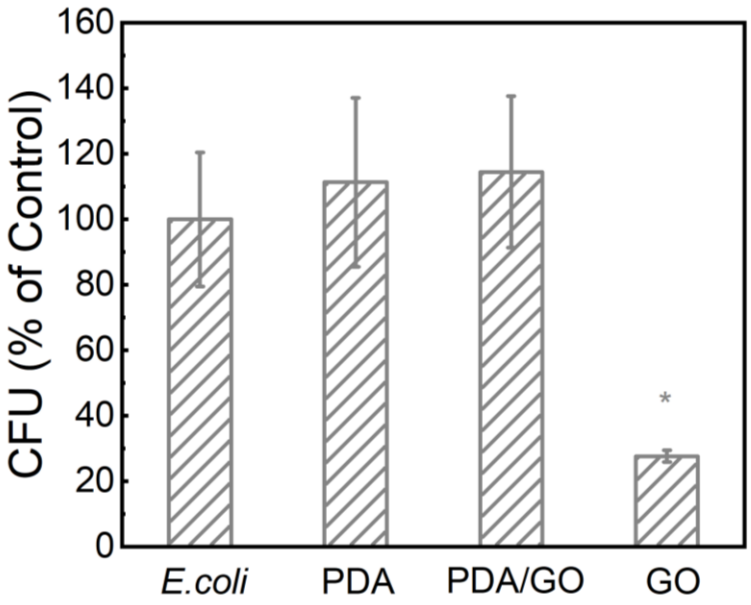

Figure S8. Antimicrobial activity test via plate counting assay of the pure PDA, pure GO, and PDA/GO mixture. After 30 minutes shaken, *E. coli* (10^8 CFU mL^{-1}) was added into the three suspensions for 3 hours contact. The colony forming units (CFU) data were normalized by the number obtained from the *E. coli* control panel. The symbol "*" denotes the statically significant difference between the *E. coli* control panel and the pure GO sample (Student's t-test, n=3, $p<0.05$).

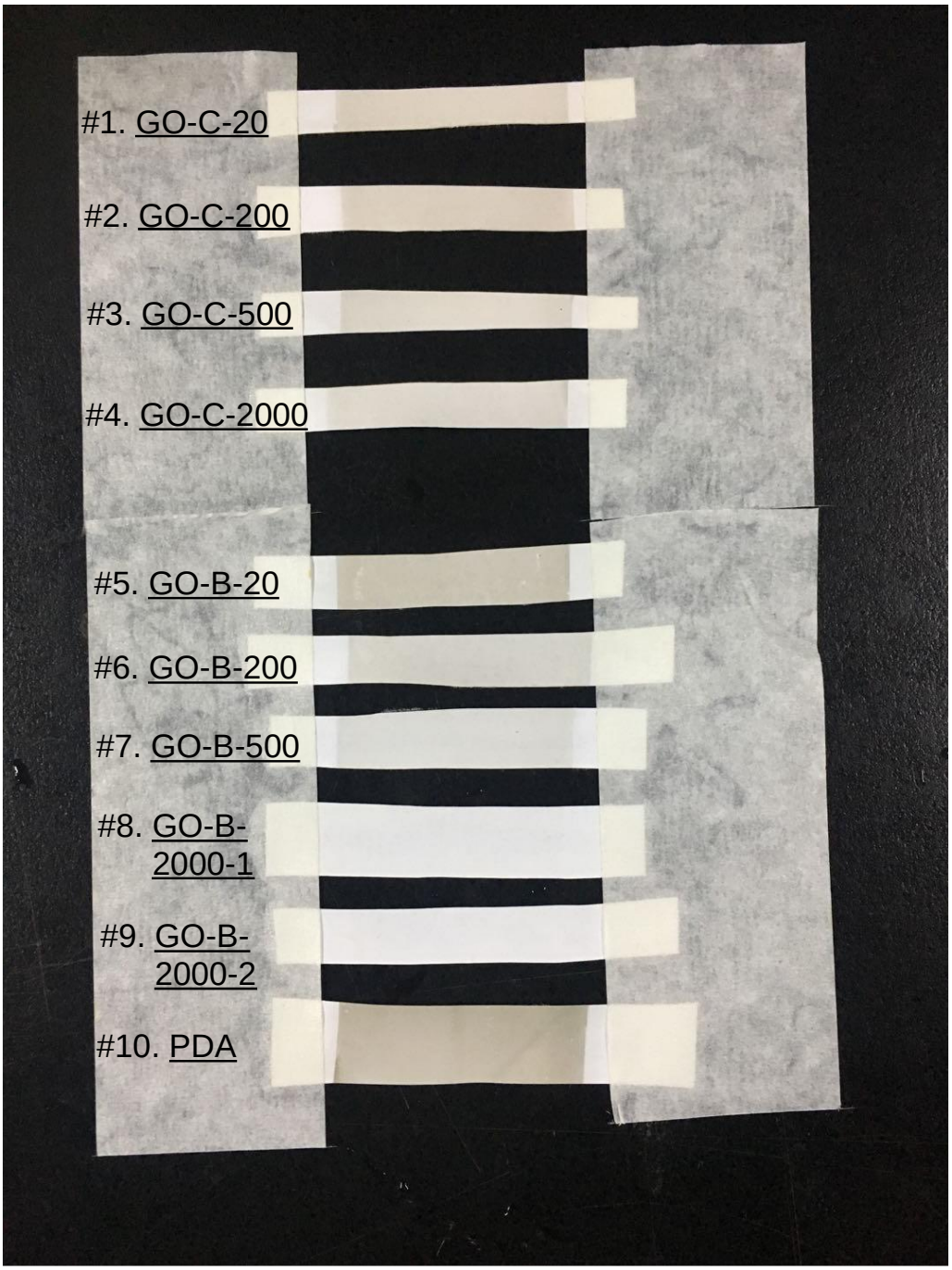

Figure S9. Photograph of the GO-functionalized membranes with various GO concentrations. The detail of PDA/GO compositions of modified membranes is provided in Table S2.

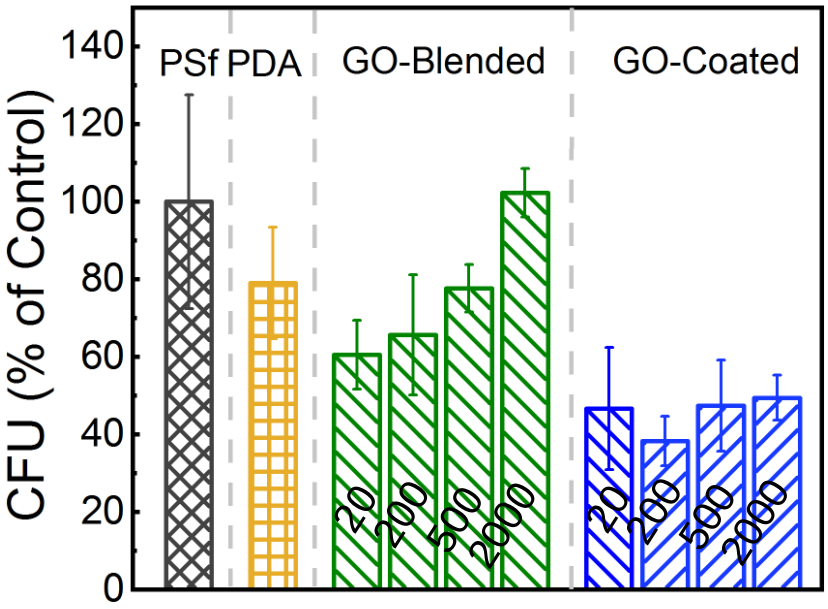

Figure S10. Antimicrobial activity of the polysulfone, PDA-only, GO-blended, and GO-coated membranes modified with various GO concentration.

Table S1. Feed solution composition for biofouling experiment.

	Component	Concentration
Ionic salts	$NaHCO_3$	0.5 mM
	NH_4Cl	0.4 mM
	$CaCl_2$	0.2 mM
	$MgSO_4$	0.15 mM
	NaCl	8 mM
	KH_2PO_4	0.2 mM
Carbon source	Glucose	0.6 mM
Bacterium	*E. coli*	3×10^6 CFU mL^{-1}

Table S2. PDA/GO composition of the modified membranes

No.	Membrane	GO concentration ($mg\ L^{-1}$)	Dopamine concentration ($g\ L^{-1}$)
#1	GO-Coated-20	20	2
#2	GO-Coated-200	200	2
#3	GO-Coated-500	500	2
#4	GO-Coated-2000	2000	2
#5	GO-Blended-20	20	2
#6	GO-Blended-200	200	2
#7	GO-Blended-500	500	2
#8	GO-Blended-2000-1	2000	2
#9	GO-Blended-2000-2	2000	10
#10	PDA only	/	2

References

1. Liu, C.; Faria, A. F.; Ma, J.; Elimelech, M., Mitigation of biofilm development on thin-film composite membranes functionalized with zwitterionic polymers and silver nanoparticles. *Environ. Sci. Technol.* **2017,** 51, 182-191.
2. Xie, M.; Bar-Zeev, E.; Hashmi, S. M.; Nghiem, L. D.; Elimelech, M., Role of reverse divalent cation diffusion in forward osmosis biofouling. *Environ. Sci. Technol.* **2015,** 49, 13222-13229.
3. Bar-Zeev, E.; Perreault, F.; Straub, A. P.; Elimelech, M., Impaired performance of pressure-retarded osmosis due to irreversible biofouling. *Environ. Sci. Technol.* **2015,** 49, 13050-13058.
4. Perreault, F.; Tousley, M. E.; Elimelech, M., Thin-film composite polyamide membranes functionalized with biocidal graphene oxide nanosheets. *Environ. Sci. Technol. Lett.* **2014,** 1, 71-76.