Supporting Information

Antimicrobial activity of thin-film composite membranes functionalized with cellulose nanocrystals and silver nanoparticles via one-pot deposition and layer-by-layer assembly

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Supporting information contains 16 pages with 2 sections: in the first section the detailed description of the materials and methods utilized in this work is provided; the second section contains the supplementary figures and tables.

SECTION 1: ADDITIONAL METHODS

Confocal Microscopy of *E. coli* on Membranes. Briefly, membrane coupons were placed in PVC holders and contacted with a suspension of *E. coli* at 10^8 CFU/mL for 3 h at room temperature using 3 mL of bacterial suspension per cm^2 of exposed membrane surface. The bacterial suspension was prepared following the methods described in the main manuscript. Membranes were then washed with sterile saline to remove loosely attached cells and some membrane samples were prepared for fluorescence imaging via confocal microscopy. Following the saline wash, 5 mL of fresh, sterile LB was applied to the membrane surface. The membranes in the PVC holders were incubated overnight in static conditions at 37° C to encourage the growth of the well-attached bacteria on the membrane surface into a biofilm. After incubation, the LB was discarded, and the membranes were stained with SYTO 9 to label live cells and propidium iodide (PI) to label dead cells. Stained membranes were imaged in a Nikon A1R MP confocal microscope.

SECTION 2: SUPPLEMENTARY FIGURES

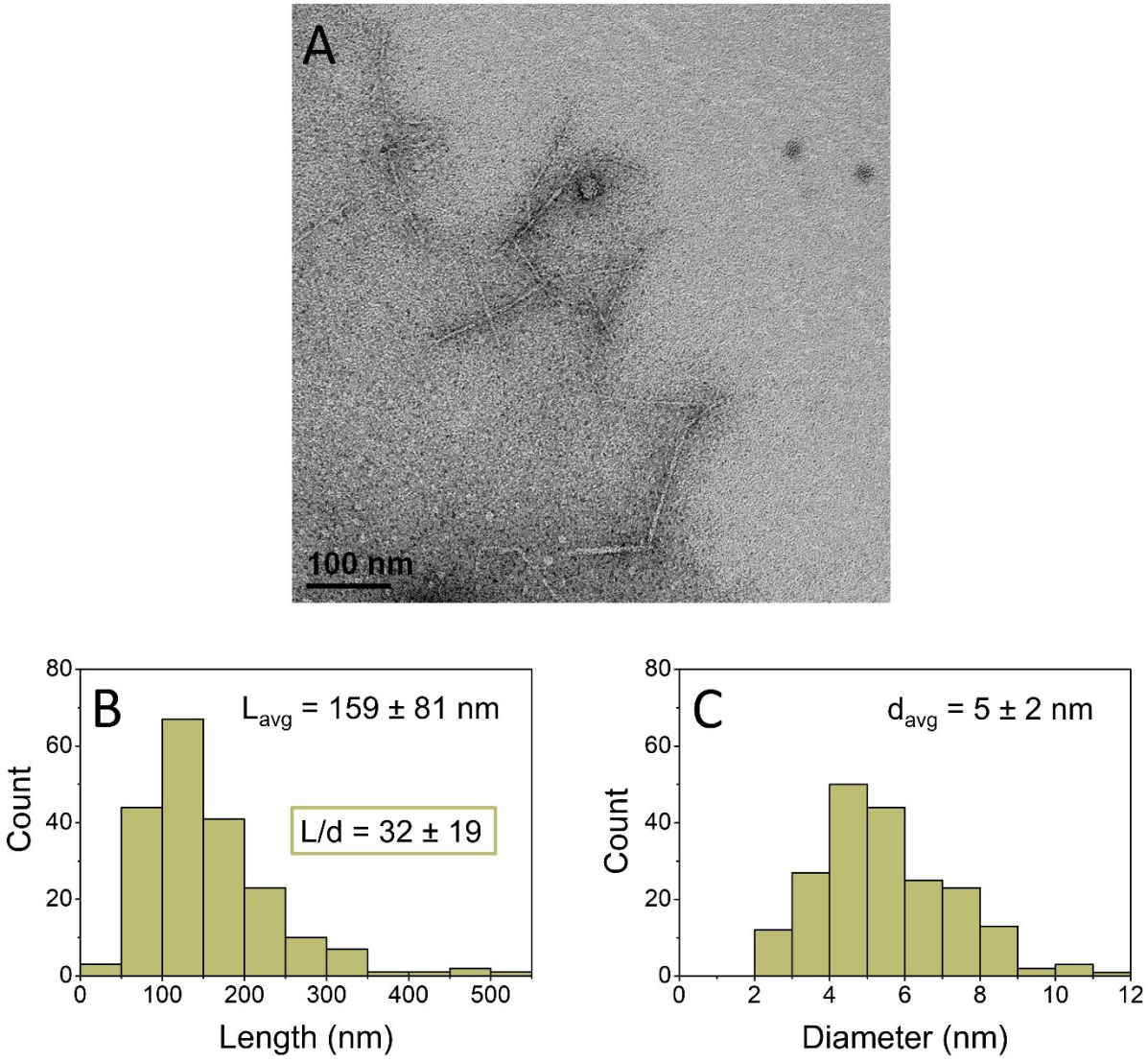


Figure S1. (A) TEM image of cellulose nanocrystals obtained by TEMPO-oxidation followed by sonication of a cellulose-rich substrate isolated from elephant grass leaves. Scale bar: 100 nm. Size distribution histograms of (B) length and (C) diameter measured for 200 nanoparticles in ten different images. The average length and diameter calculated were 159 ± 81 nm and 5 ± 2 nm, respectively. The aspect ratio (length/diameter) was 32 ± 19.

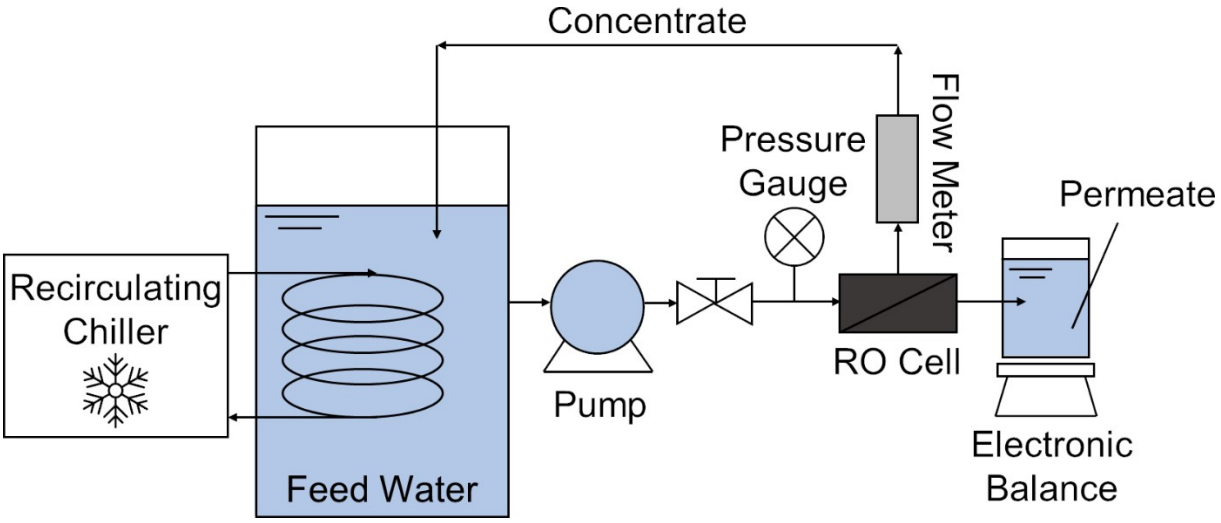


Figure S2. Schematic of the bench-scale reverse osmosis membrane filtration system.

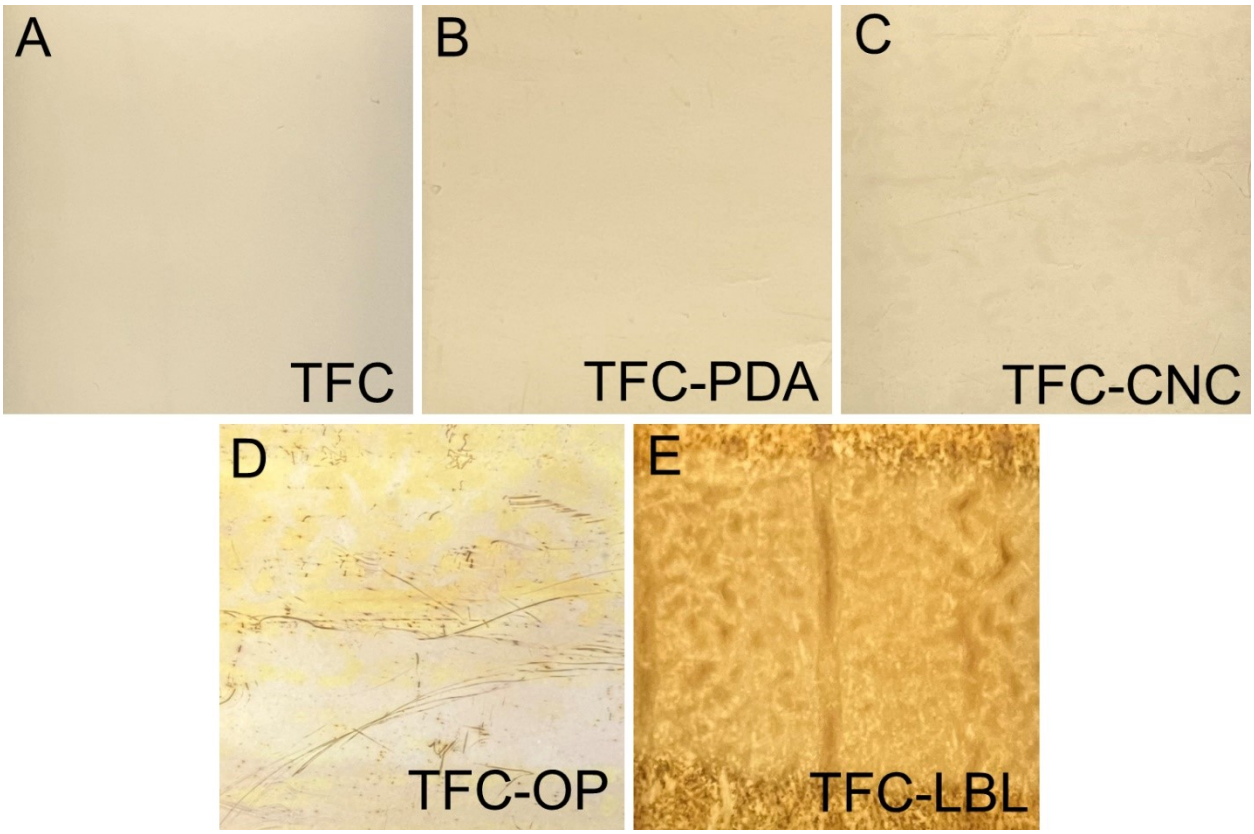


Figure S3. Photographs of the (A) pristine TFC membrane, (B) membrane modified with only PDA (TFC-PDA), (C) membrane modified with CNC using PDA (TFC-CNC), (D) membrane modified using the "one-pot" method to attach CNC/Ag to the membrane surface with PDA (TFC-OP), and (E) membrane modified using the "layer-by-layer" method to attach CNC to the membrane via PDA followed by *in situ* AgNP formation (TFC-LBL).

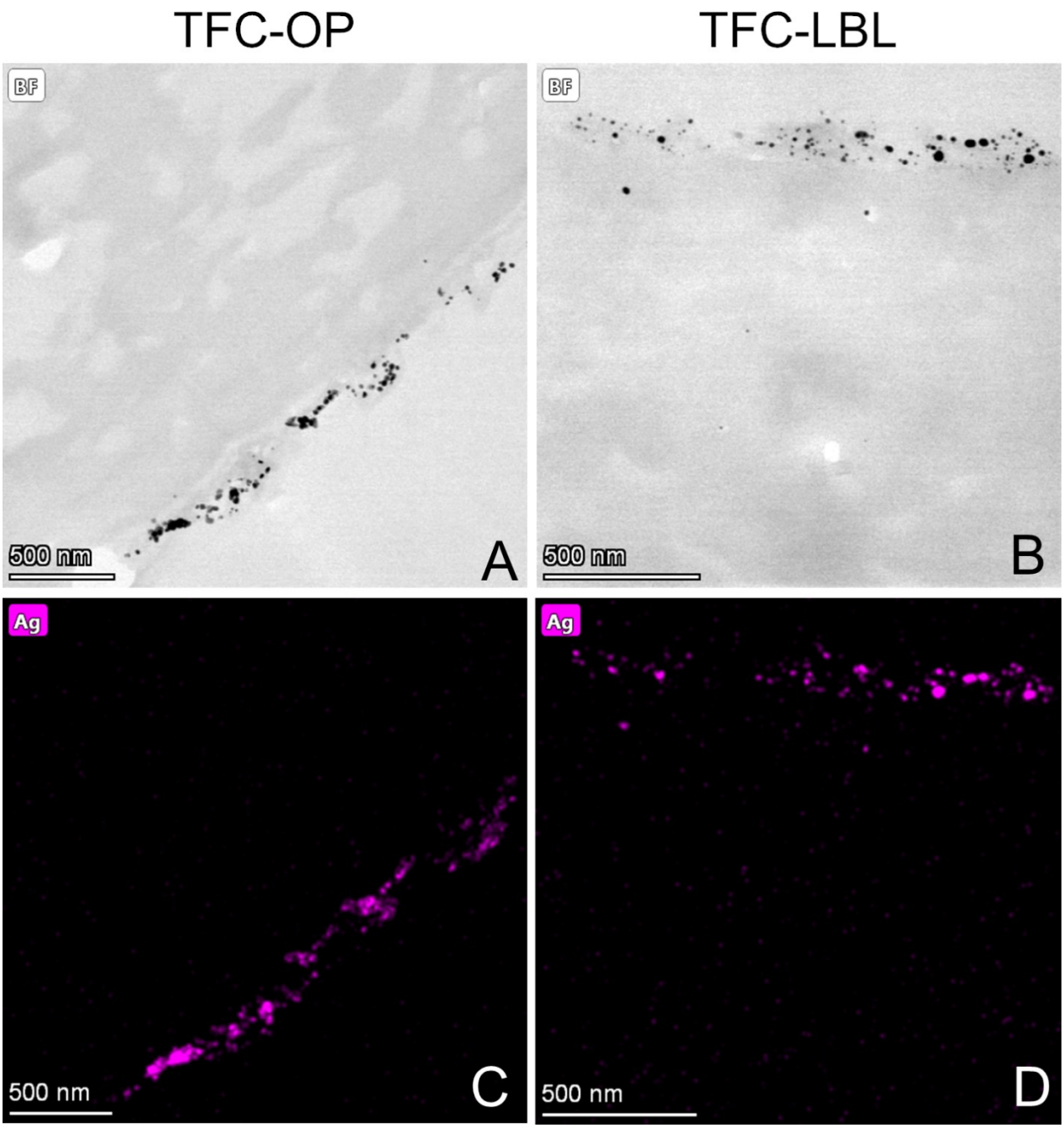


Figure S4. Cross-sectional TEM of the (A) TFC-OP membrane (B) TFC-LBL membrane. In (A) and (B), the AgNP can be observed as black dots at the membrane surface. EDS analysis was used to confirm the presence of Ag in these membranes which can be seen indicated by a pink color (C, D).

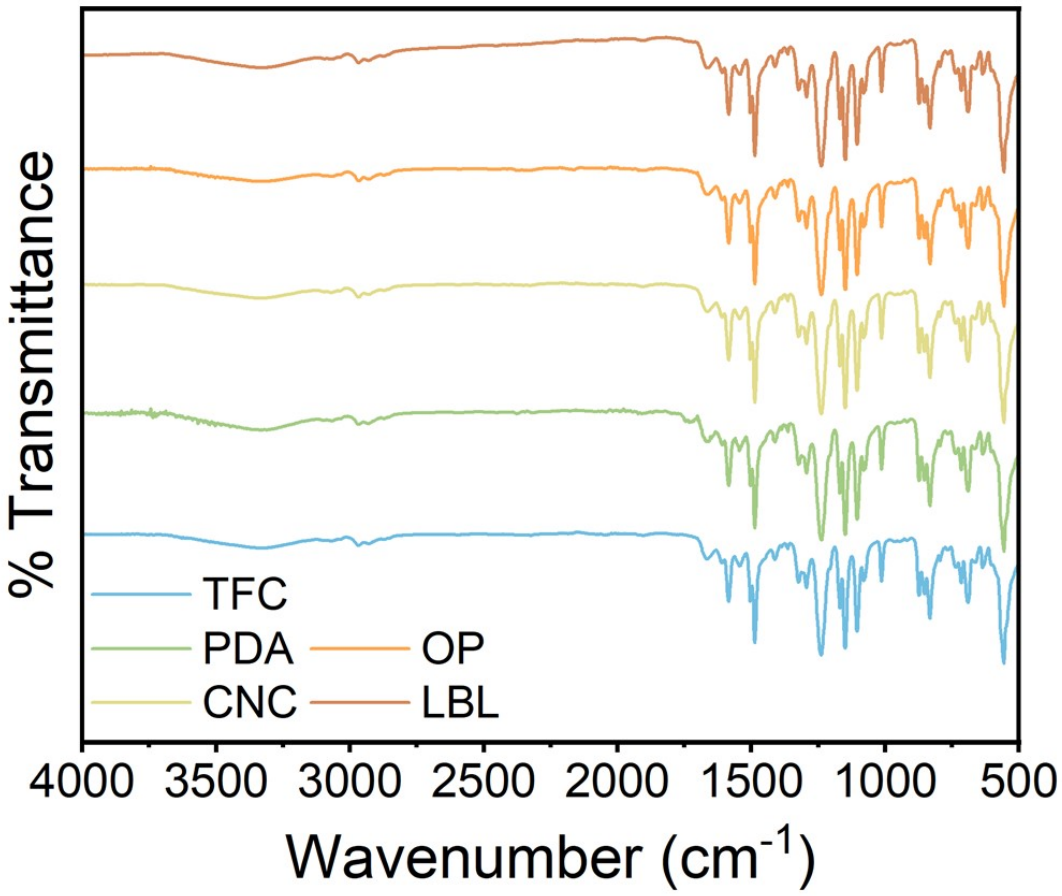


Figure S5. FTIR spectra for the pristine and functionalized membranes.

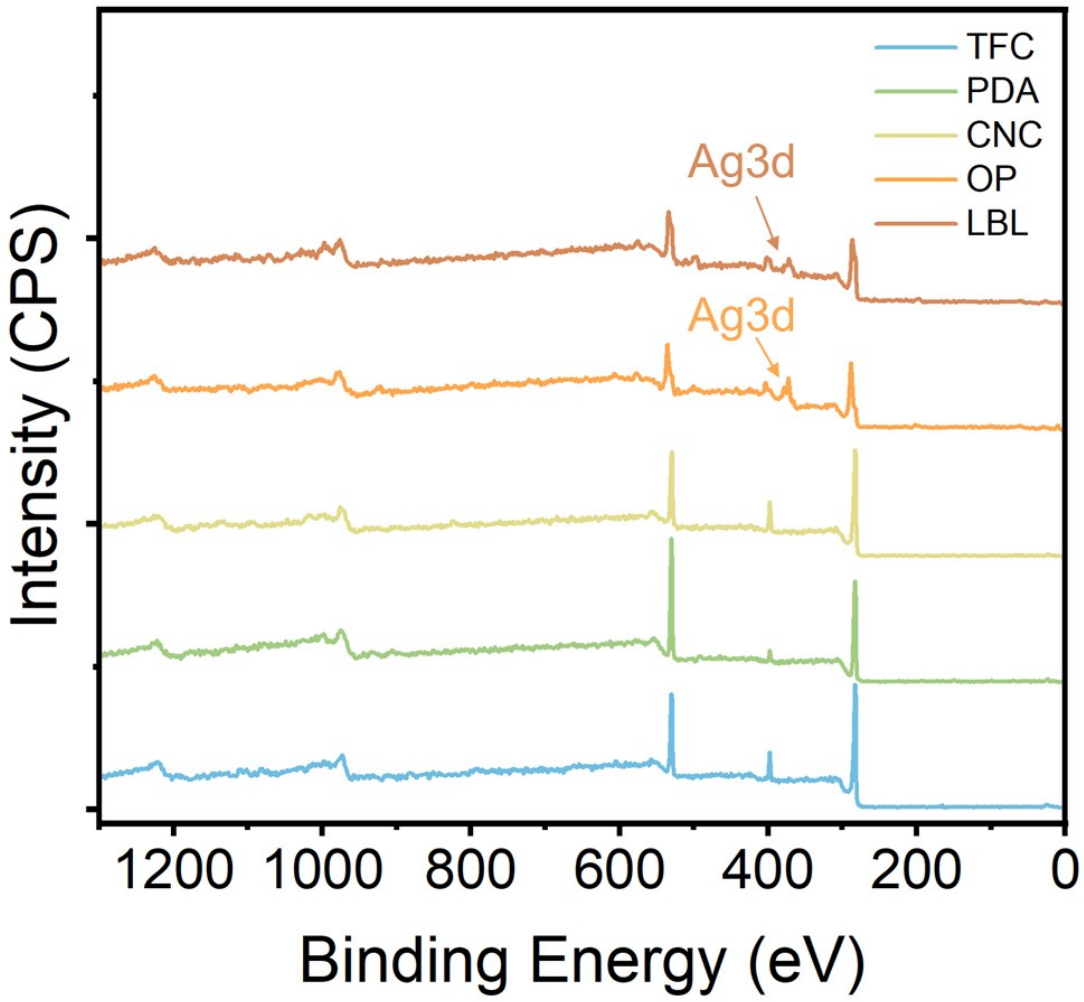
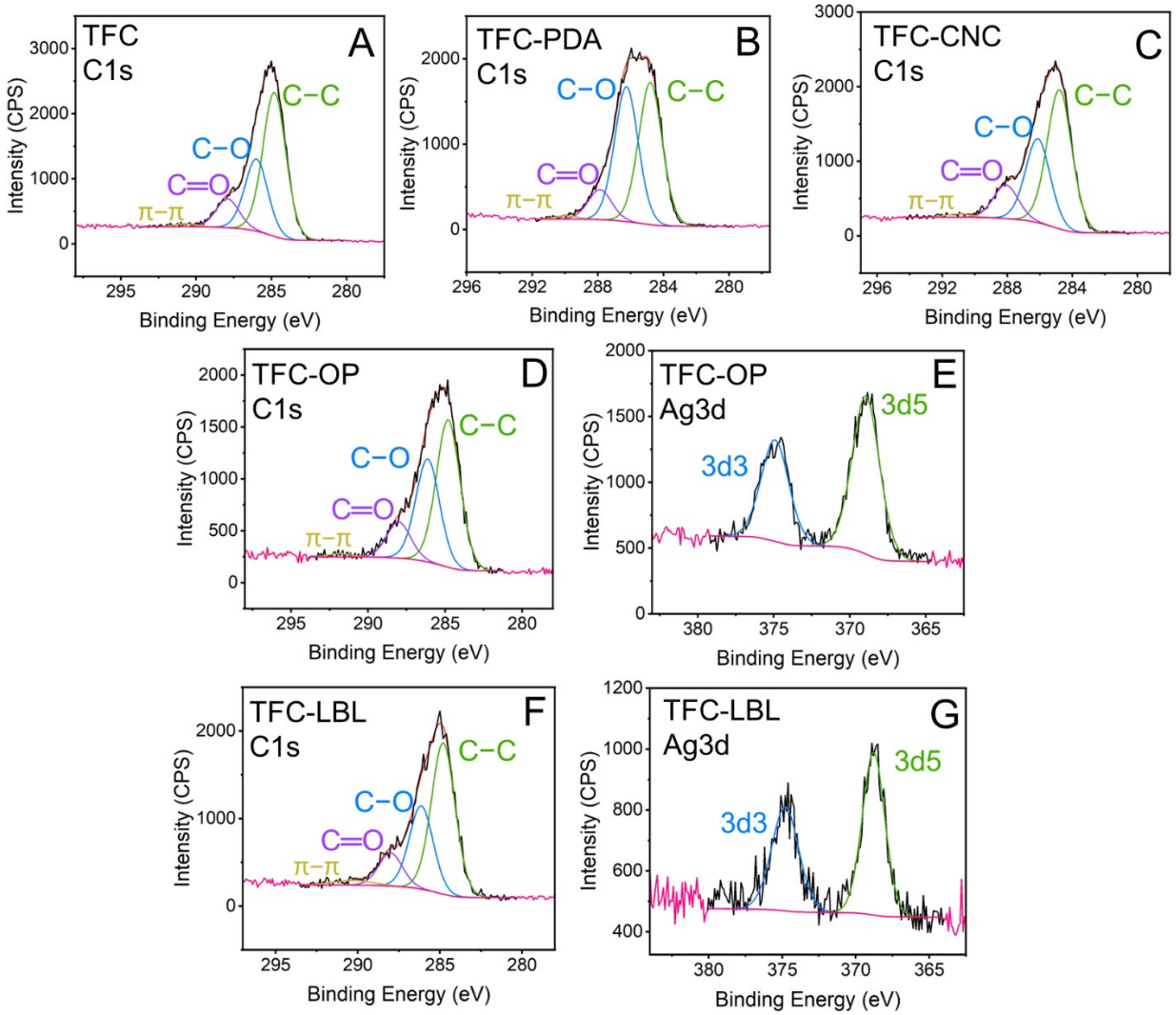


Figure S6. XPS full survey spectra for the pristine and functionalized membranes.



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83 **Figure S7.** High-resolution C 1s spectra for the pristine TFC (A), TFC-PDA (B), and TFC-CNC

84 (C) membranes. High-resolution spectra for the TFC-OP membrane for C 1s (D) and Ag 3d (E).

85 High-resolution spectra for the TFC-LBL membrane for C 1s (F) and Ag 3d (G).

Table S1. Summary of relative peak intensities from XPS analysis of TFC membranes modified with CNC and AgNP using PDA.

Element	Binding Energy (eV)	Bond	% Area TFC	% Area PDA	% Area CNC	% Area OP	% Area LBL
C 1s	284.8	C−C	58.10	45.78	53.44	50.80	54.86
C 1s	286.0	C−O	29.10	43.51	32.26	35.47	30.19
C 1s	287.9	C═O	11.68	9.66	12.95	12.50	12.05
C 1s	290.7	π−π	1.12	1.05	1.35	1.23	2.90
Ag 3d	368.9	Ag 3d5	N/A	N/A	N/A	60.56	56.54
Ag 3d	374.9	Ag 3d3	N/A	N/A	N/A	39.44	43.46

89 **Table S2**. Overview of key parameters impacted by different approaches to functionalize membranes with AgNP.

Strategy to obtain the silver-containing membrane	Change in water permeability coefficient	Change in salt permeability	Silver loading	Silver leaching rate	Antimicrobial performance	Reference
"One-pot" immobilization of CNC/Ag hybrid	+11%	+89%	1.71 μg cm^{-2}	0.027 μg cm^{-2} day^{-1}	75.7% inactivation of *E. coli*	This work
"Layer-by-layer" deposition of CNC and *in situ* nucleation of AgNP	+16%	+494%	3.55 μg cm^{-2}	0.091 μg cm^{-2} day^{-1}	90.1% inactivation of *E. coli*	This work
AgNP covalently bonded to polyamide active layer	+32%	+2%	15.5 μg cm^{-2}	0.1 μg cm^{-2} day^{-1}	Good inhibition against *E. coli*	(Yin et al., 2013)
Covalent immobilization of AgNP-decorated silica particles	–3%	+0.2%	0.155 μg cm^{-2}	0.0011 μg cm^{-2} day^{-1}	92.7% (*E. coli)*, 99.5% (*P. aeruginosa*), 73.3% (*S. aureus*)	(Park et al., 2015)
In situ generation of AgNP followed by interfacial polymerization	+210%	–7%	14.7 μg cm^{-2}	13.1 μg L^{-1} (initial release)	44.4% (*E. coli)* and 90.1% (*B. subtilis*)	(Yang et al., 2017)
Covalent immobilization of graphene oxide/AgNP	–10%	+19%	-	-	80% inactivation of *P. aeruginosa*	(Faria et al., 2017)
Embedding of graphene oxide quantum dot/AgNP via interfacial polymerization	+44%	–0.3%	Up ~4 μg mL^{-} (accumulative release)	~0.75 μg mL^{-2} day^{-1}	98.6% (*E. coli*) and 96.5% (*S. aureus*)	(Yu et al., 2019)
Grafting zwitterionic polymer brushes and AgNP	–31%	+103%	~7.5 μg cm^{-2}	~0.5 μg cm^{-2} day^{-1}	97% inactivation of *P. aeruginosa*	(Liu et al., 2016)
Addition via interfacial polymerization of tannic acid-functionalized carbon nanotubes embedded with AgNP	+15%	+0.1%	2.29 μg cm^{-2}	0.014 μg L^{-1} (initial release)	97.8% inactivation of *E. coli*	(Zhao et al., 2021)

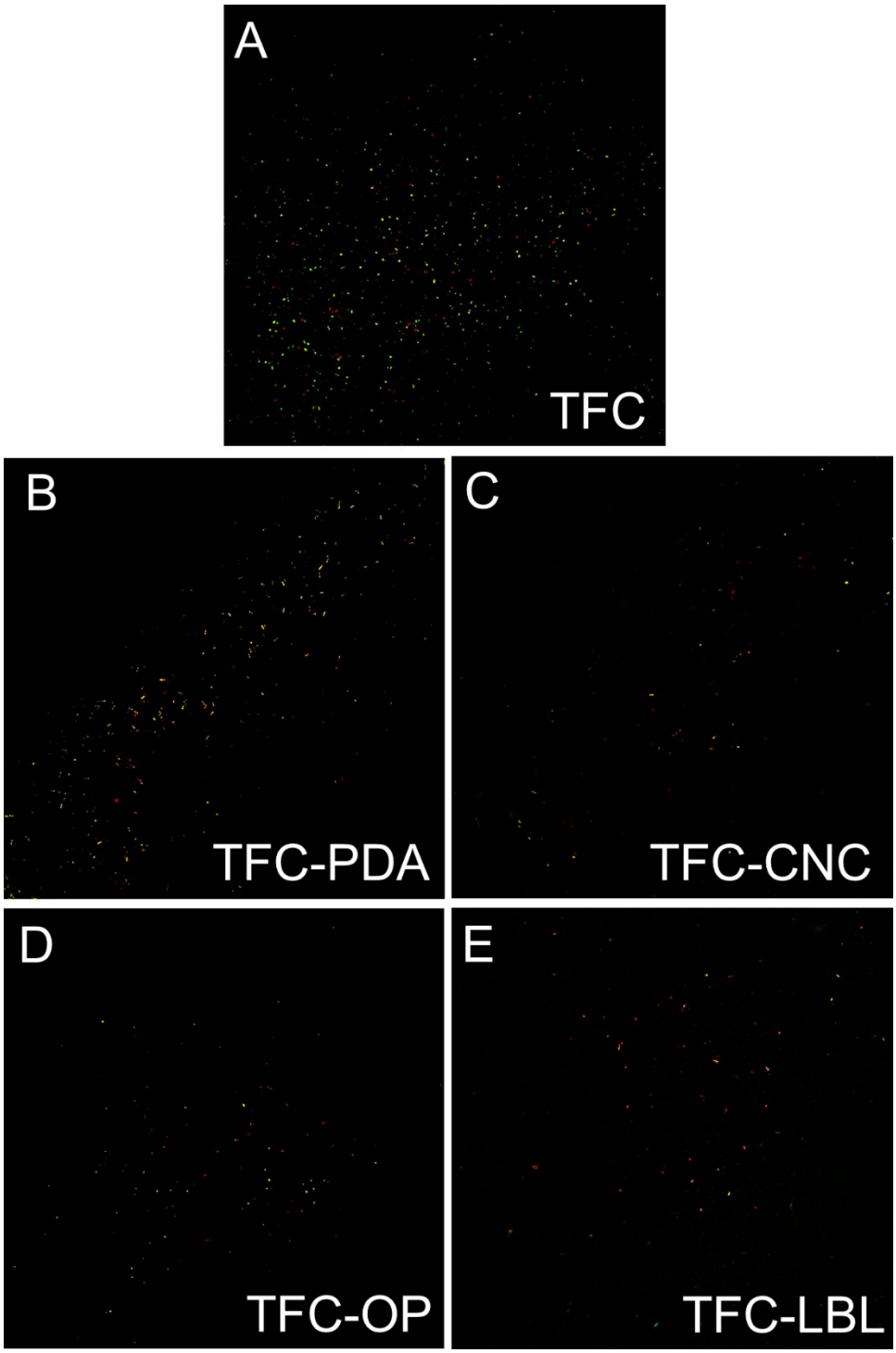

Figure S8. Confocal images showing *E. coli* cells on the membrane surface following the static biofouling assay. Live cells stained with SYTO 9 are shown in green while dead cells stained with PI are shown in red.

Table S3. Live and dead cells counted from confocal images taken following the static biofouling assay in darkness.

Sample	Total Cells	Live Cells (%)	Dead Cells (%)
TFC	253	59.7	40.3
TFC-PDA	119	32.8	67.2
TFC-CNC	22	50.0	50.0
TFC-OP	42	28.6	71.4
TFC-LBL	34	26.5	73.5

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