Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

A tethered bilayer lipid membrane that mimicks microbial membranes

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APPENDIX 1: NEUTRON DATA

Table S 1: All parameters of fully tethered bilayers and bilayers with 10 mg/mL Colistin. SLD give in 10-6 Å-2, Thickness and roughness given in Å and hydration given in volume-%.

	100% DPhyTL, 100% RcLPS		100% DPhyTL, 98% RcLPS		100% DPhyTL, 94% RcLPS	
	Bilayer	Bilayer + 10 mg/mL Colistin	Bilayer	Bilayer + 10 mg/mL Colistin	Bilayer	Bilayer + 10 mg/mL Colistin
Scale factor	1.09 ± 0.01	1.00	0.88 ± 0.01	0.93	0.89 ± 0.01	0.92
fronting SLD	2.07	2.07	2.07	2.07	2.07	2.07
backing roughness	3.64 ± 0.51	6.26	5.79 ± 1.27	6.88	5.58 ± 1.28	7.55
SiO_2 thickness	27.2 ± 1.35	20.0	38.2 ± 0.73	41.88	35.6 ± 0.81	32.76
SiO_2 SLD	3.47	3.47	3.47	3.47	3.47	3.47
SiO_2 hydration	0.00	0.00	0.00	0.00	0.00	0.00
SiO_2 roughness	3.42 ± 0.33	4.90	3.21 ± 0.17	3.02	3.84 ± 0.61	5.64
Cr thickness	30.2 ± 1.60	35.34	5.12 ± 0.10	6.32	5.17 ± 0.15	5.01
Cr SLD	3.03	3.03	3.02 ± 0.01	3.01	3.02 ± 0.01	3.02
Cr hydration	0.00	0.00	0.00	0.00	0.00	0.00
Cr Roughness	4.40 ± 1.01	7.91	3.39 ± 0.32	4.65	3.64 ± 0.51	6.49
Cr Oxide thickness*	45.7 ± 1.58	46.71	*These layers are not present in the other samples.			
Cr Oxide SLD*	3.85 ± 0.03	3.88				
Cr Oxide hydration*	0.00	0.00				
Cr Oxide roughness	4.68 ± 1.09	3.92				
Cr Thickness*	38.3 ± 1.09	38.73				
Cr SLD*	3.03	3.03				
Cr hydration*	0.00	0.00				
Cr Roughness*	7.13 ± 0.76	7.04				
Au thickness*	210.46 ± 1.03	208.60	107.0 ± 0.83	102.42	231.4 ± 0.69	233.20
Au SLD	4.33 ± 0.01	4.32	4.35 ± 0.01	4.40	4.48 ± 0.01	4.49
Au hydration	0.00	0.00	0.00	0.00	0.00	0.00
Au roughness	7.90 ± 0.09	6.26	9.95 ± 0.04	9.79	11.9 ± 0.05	11.66
Tether thickness	14.02 ± 1.13	14.16	12.2 ± 0.18	12.40	12.53 ± 0.37	12.08
Tether SLD	0.98 ± 0.30	1.35	1.15 ± 0.14	1.24	0.69 ± 0.13	1.20
Tether hydration	6.41 ± 1.02	5.82	9.26 ± 0.59	6.90	5.57 ± 1.55	3.98
Tether roughness	6.43 ± 0.94	3.85	3.21 ± 0.16	3.52	4.30 ± 0.22	4.56
Inner HC thickness	15.9 ± 1.23	16.43	12.3 ± 0.22	12.32	13.1 ± 0.59	13.59
Inner HC SLD	-0.31 ± 0.06	-0.21	-0.34 ± 0.04	-0.31	-0.31 ± 0.06	-0.38
Inner HC hydration	2.06 ± 1.06	3.61	5.48 ± 0.43	4.85	1.26 ± 0.87	0.80
Inner HC roughness	5.93 ± 1.48	3.82	4.61 ± 0.96	3.63	6.19 ± 1.27	6.20
Outer HC thickness	15.9 ± 1.22	16.32	12.3 ± 0.19	12.50	12.8 ± 0.50	12.35
Outer HC SLD	-0.34 ± 0.04	-0.40	-0.05 ± 0.04	0.01 ± 0.05	0.12 ± 0.07	-0.05 ± 0.04
Outer HC hydration	2.19 ± 0.79	0.08 ± 0.06	4.13 ± 0.69	4.52 ± 0.39	2.78 ± 1.78	1.35 ± 0.93
Outer HC roughness	6.57 ± 1.79	7.66 ± 1.53	6.59 ± 1.77	6.97 ± 1.87	6.73 ± 1.52	6.82 ± 1.81
Outer HG thickness	8.40 ± 0.30	8.03 ± 0.00	9.35 ± 0.84	8.12 ± 0.09	7.29 ± 0.85	8.46
Outer HG SLD	2.83 ± 0.36	2.13 ± 0.08	3.21 ± 0.35	2.17 ± 0.15	3.35 ± 0.40	2.26 ± 0.10
Outer HG hydration	60.2 ± 3.68	10.6 ± 0.47	72.8 ± 1.73	34.1 ± 0.73	69.3 ± 3.92	24.5 ± 2.68

Outer HG roughness	3.35 ± 0.25	3.09 ± 0.08	6.83 ± 0.46	6.93 ± 0.58	4.55 ± 0.37	3.63 ± 0.43

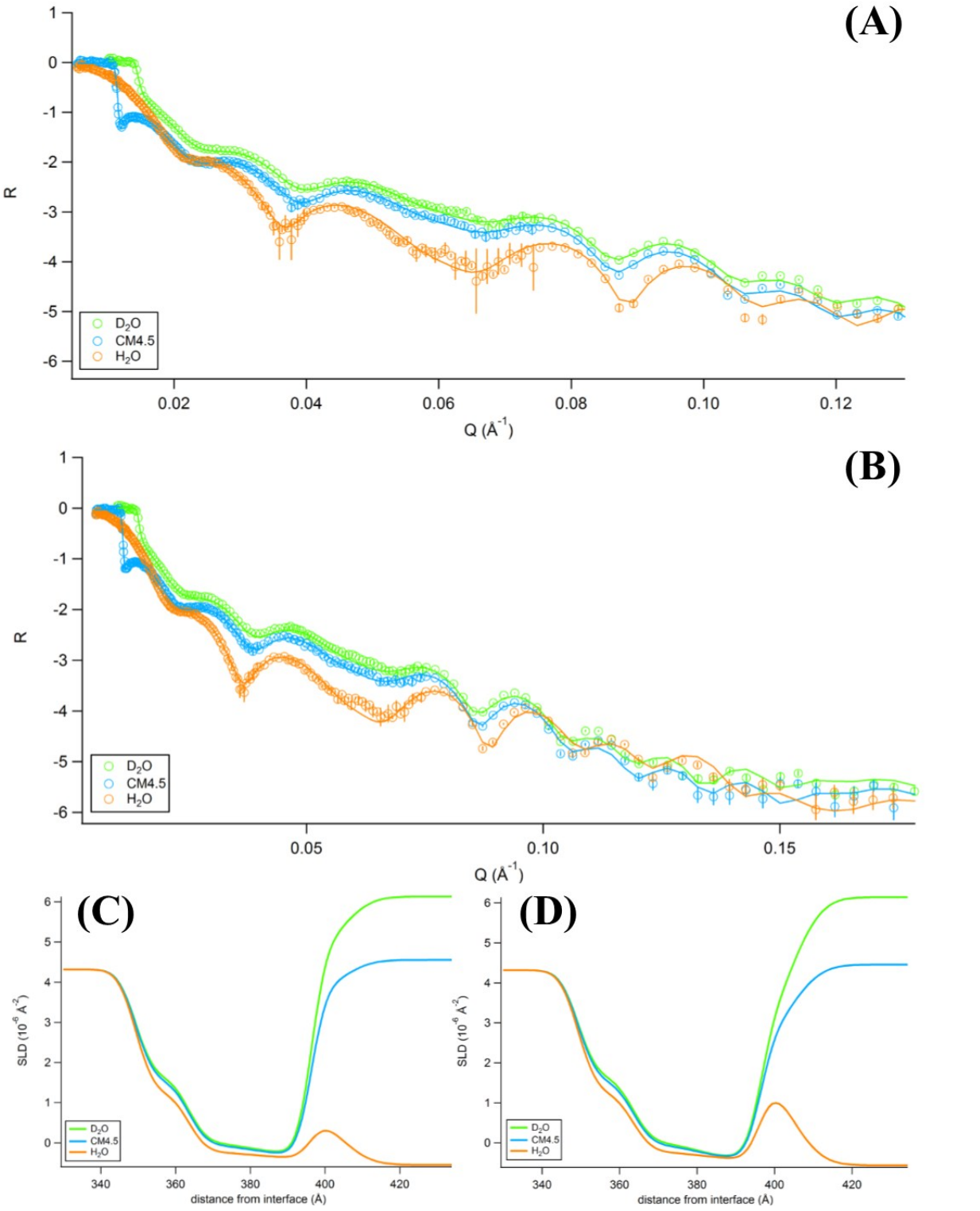

Figure S 1: Reflectivity plot of bilayer (A), bilayer after exposure to 10 mg/mL Colistin (B), SLD plot of bilayer (C) and SLD plot of bilayer after exposure to 10 mg/mL Colistin (D) for the sample at 100% DPhyTL and RcLPS.

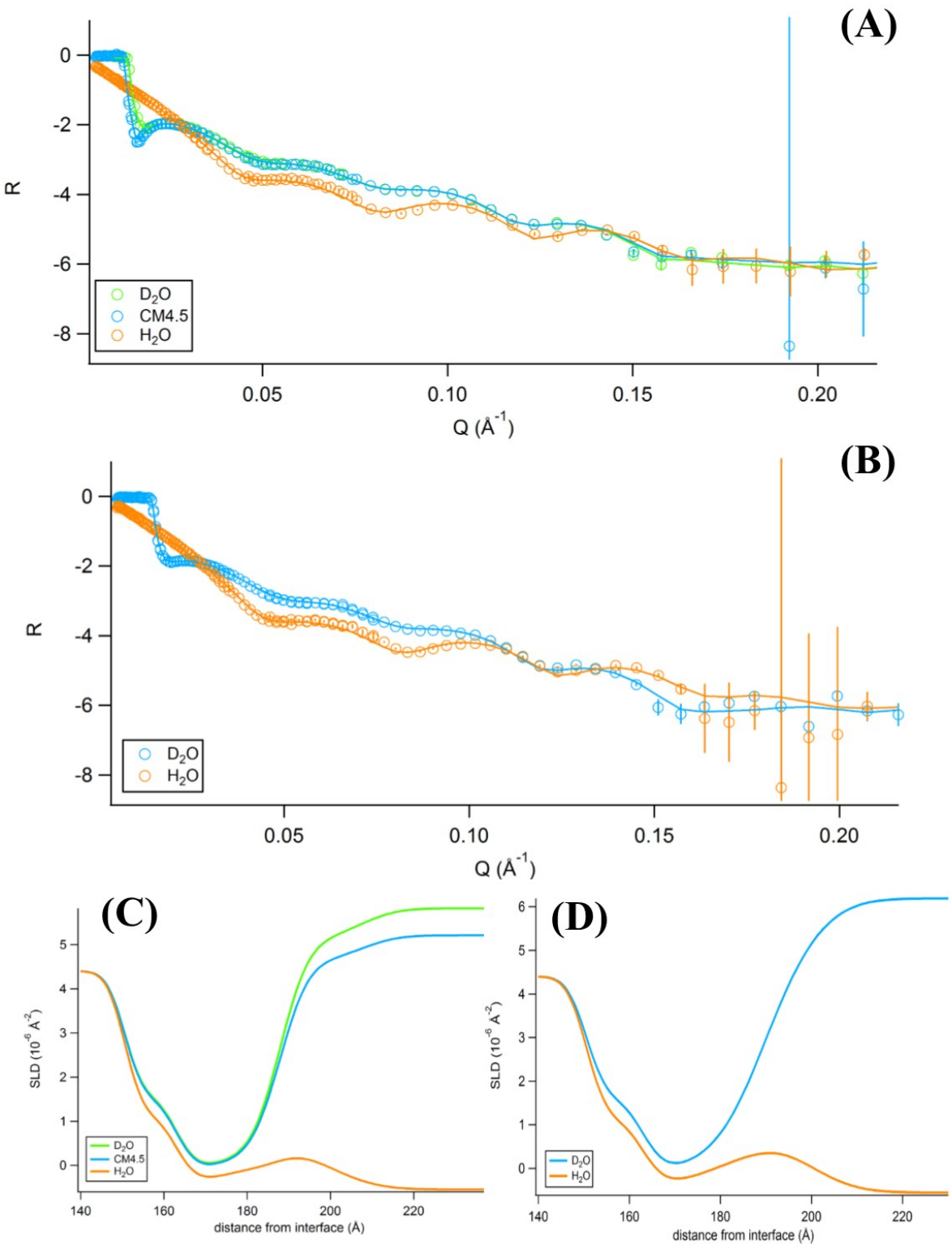

Figure S 2: Reflectivity plot of bilayer (A), bilayer after exposure to 10 mg/mL Colistin (B), SLD plot of bilayer (C) and SLD plot of bilayer after exposure to 10 mg/mL Colistin (D) for the sample at 100% DPhyTL and 98% RcLPS.

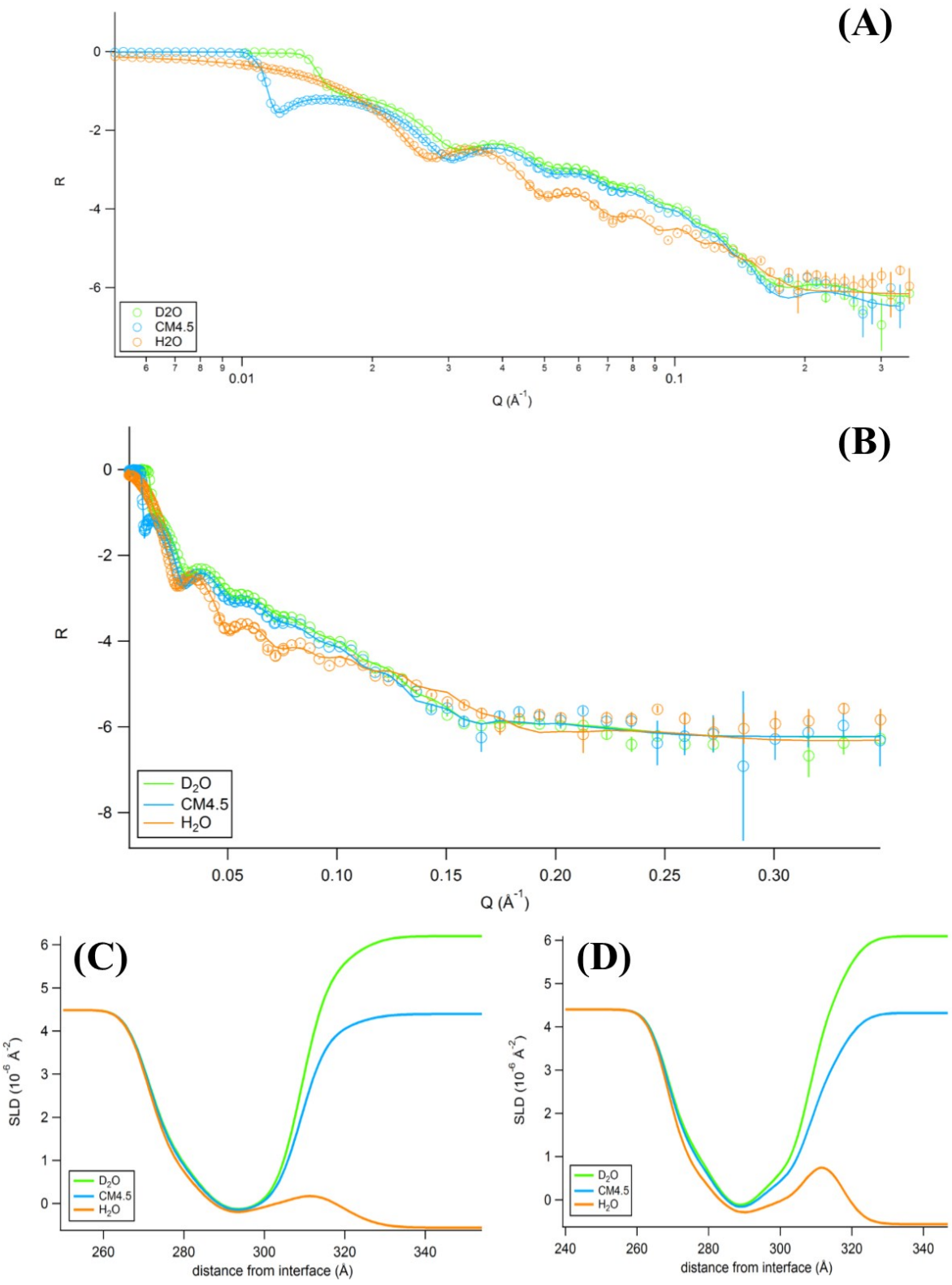

Figure S 3: Reflectivity plot of bilayer (A), bilayer after exposure to 10 mg/mL Colistin (B), SLD plot of bilayer (C) and SLD plot of bilayer after exposure to 10 mg/mL Colistin (D) for the sample at 100% DPhyTL and 94% RcLPS.

Table S 2: All parameters of sparsely tethered bilayers and bilayers with 10 mg/mL Colistin. SLD given in 10-6 Å-2, Thickness and roughness given in Å and hydration given in volume-%.

	80% DPhyTL, 98% RcLPS		**80% DPhyTL, 94% RcLPS**	
	Bilayer	**Bilayer + 10 mg/mL Colistin**	**Bilayer**	**Bilayer + 10 mg/mL Colistin**
scale factor	0.96 ± 0.01	0.93	0.93 ± 0.01	0.90 ± 0.01
fronting SLD	2.07	2.07	2.07	2.07
backing SLD	6.23 ± 0.01	6.15	6.24 ± 0.01	6.15 ± 0.02
backing roughness	7.71 ± 0.25	7.36	6.44 ± 1.07	6.06
SiO2 thickness	45.5 ± 0.65	40.76	48.5 ± 0.60	44.93
SiO2 SLD	3.47	3.47	3.47	3.47
SiO2 hydration	0	0	0	0
SiO2 roughness	3.49 ± 0.38	3.09	4.35 ± 0.83	3.99
Cr thickness	5.22 ± 0.17	6.96	5.38 ± 0.30	9.12
Cr SLD	3.02 ± 0.01	3.02	3.02 ± 0.01	3.02
Cr hydration	0	0	0	0
Cr Roughness	3.47 ± 0.38	3.86	5.58 ± 0.47	6.65
Au thickness	239.2 ± 0.73	243.21	226.5 ± 0.92	226.49
Au SLD	4.49 ± 0.01	4.49	4.49 ± 0.01	4.46
Au hydration	0	0	0	0
Au roughness	7.93 ± 0.06	7.73	7.90 ± 0.08	7.9
Tether thickness	8.33 ± 0.25	8.11	6.38 ± 0.29	6.65
Tether SLD	0.96 ± 0.25	0.59	1.12 ± 0.24	0.8
Tether hydration	11.1 ± 2.51	6.08	11.1 ± 2.32	10.72
Tether roughness	4.83 ± 0.41	5.27	4.52 ± 0.74	4.09
Inner HG thickness	6.32 ± 0.24	6.34	6.35 ± 0.27	6.62
Inner HG SLD	1.33 ± 0.28	1.19	1.64 ± 0.69	1.67
Inner HG hydration	17.4 ± 2.73	18.2	10.1 ± 3.37	7.4
Inner HG roughness	7.95 ± 1.48	6.64	7.46 ± 1.88	7.56
Inner HC thickness	10.1 ± 0.83	15.66 ± 2.67	12.6 ± 0.51	13.0 ± 0.51
Inner HC SLD	0.23 ± 0.02	0.02 ± 0.1	0.26 ± 0.05	0.04 ± 0.05
Inner HC hydration	1.76 ± 1.12	0.87 ± 0.72	2.38 ± 1.37	0.70 ± 0.63
Inner HC roughness	4.52 ± 0.42	3.83 ± 2.26	3.99 ± 0.92	0.74 ± 0.50
Outer HC thickness	15.7 ± 0.96	8.92 ± 2.80	12.6 ± 0.46	13.7 ± 0.82
Outer HC SLD	0.01 ± 0.01	0.02 ± 0.55	0.41 ± 0.13	0.68 ± 0.09
Outer HC hydration	2.24 ± 1.21	2.55 ± 2.54	2.83 ± 1.25	4.62 ± 1.73
Outer HC roughness	6.35 ± 1..44	13.8 ± 3.47	6.13 ± 1.81	18.9 ± 2.45
Outer HG thickness	7.34 ± 0.27	10.2 ± 2.81	13.8 ± 2.66	15.78 ± 0.20
Outer HG SLD	2.05 ± 0.04	1.95 ± 0.60	2.57 ± 0.53	2.33 ± 0.15
Outer HG hydration	63.8 ± 0.98	47.1 ± 18.0	78.4 ± 5.04	62.2 ± 3.21
Outer HG roughness	5.43 ± 0.51	5.37 ± 2.30	7.26 ± 0.93	4.40 ± 0.34

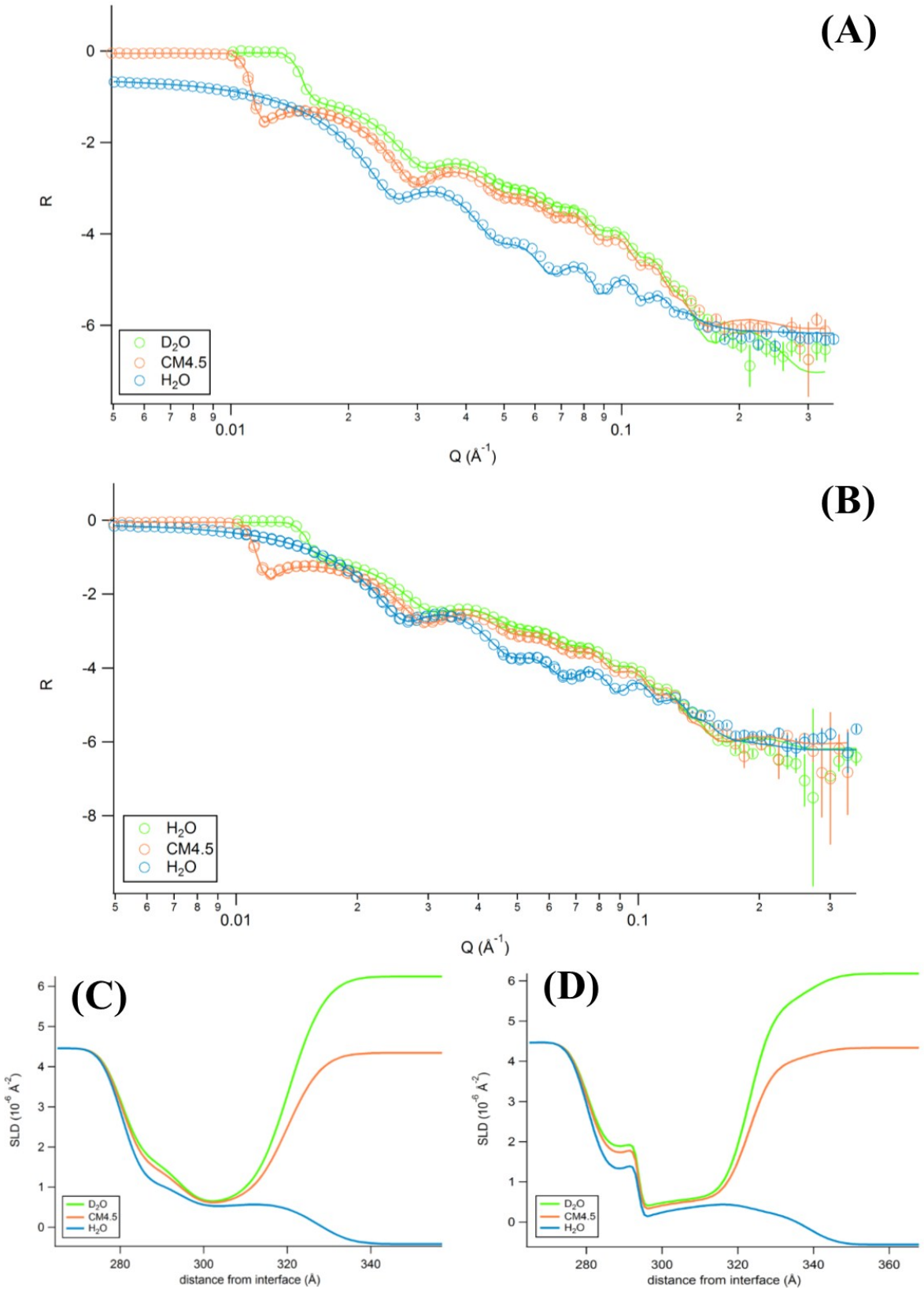

Figure S 4: Reflectivity plot of bilayer (A), bilayer after exposure to 10 mg/mL Colistin (B), SLD plot of bilayer (C) and SLD plot of bilayer after exposure to 10 mg/mL Colistin (D) for the sample at 80% DPhyTL and 98% RcLPS.

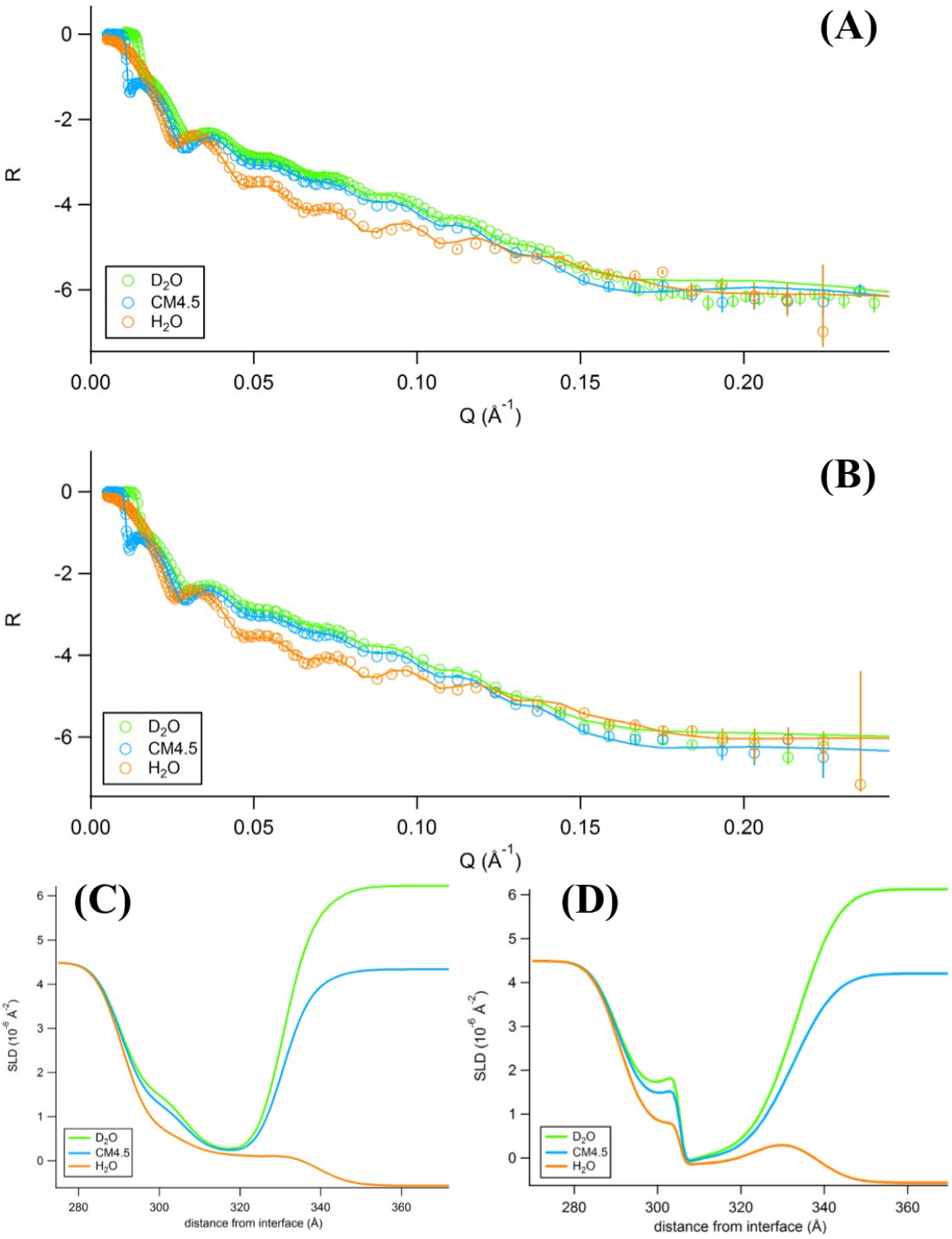

Figure S 5: Reflectivity plot of bilayer (A), bilayer after exposure to 10 mg/mL Colistin (B), SLD plot of bilayer (C) and SLD plot of bilayer after exposure to 10 mg/mL Colistin (D) for the sample at 80% DPhyTL and 94% RcLPS.

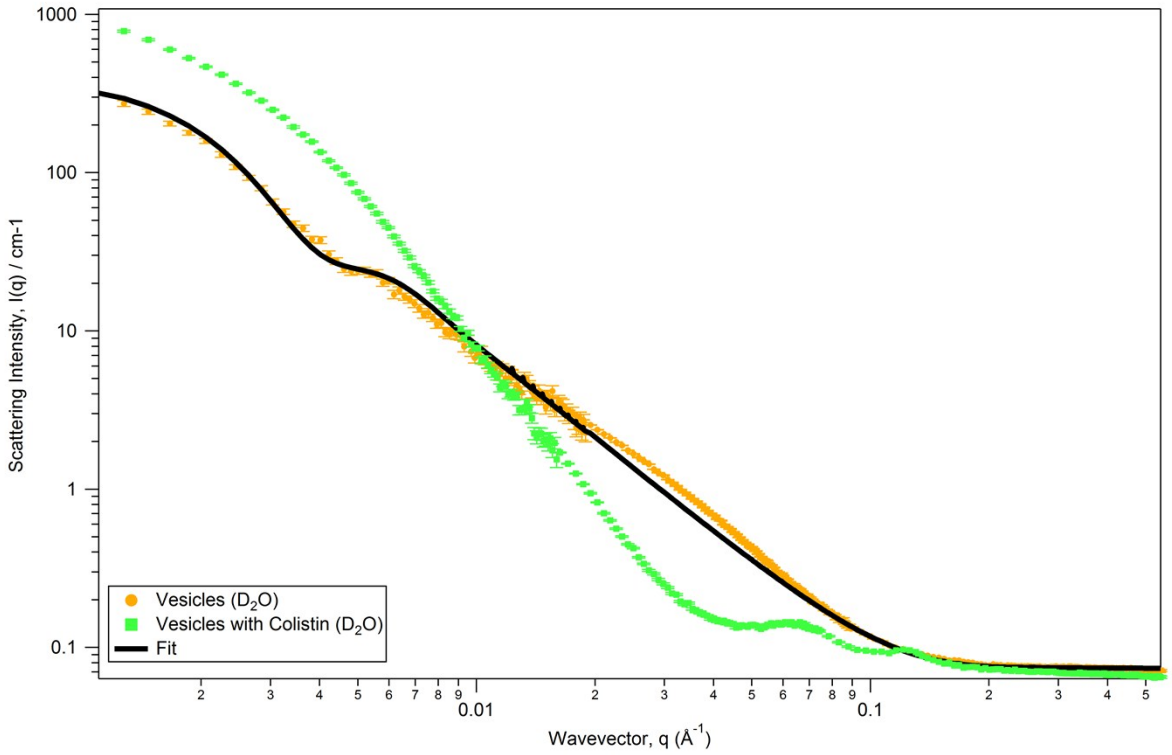

Figure S 6: Small angle neutron scattering (SANS) plot of vesicles comprised of lipopolysaccharides obtained from *Psudomonas aeruginosal* prior to (orange) and after addition of 10 mg/mL Colistin.(green) and fit (black).

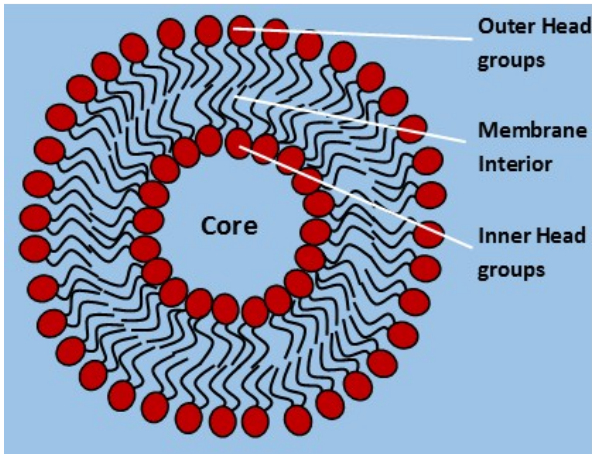

Figure S 7: Schematic of LPS model used to fit SANS data comprised of the water-filled core, the inner head groups (red), a hydrocarbon membrane interior (black) and the outer head groups (red).

Table S 3: SANS parameters of LPS-vesicles prior to exposure of Colistin. Parameter were fitted manually, therefore no error is available.

Core radius (Å)	570.0
Core polydispersity	0.295
Core SLD (10^{-6} A^{-2})	6.30
Inner head groups thickness (Å)	16
Inner head groups SLD (10^{-6} A^{-2})	4.10
Membrane interior thickness (Å)	9
Membrane interior SLD (10^{-6} A^{-2})	-0.4
Outer head groups (Å)	8
Outer head groups SLD (10^{-6} A^{-2})	3.90
Solvent SLD (10^{-6} A^{-2})	6.18
Background (cm^{-1})	7.40 x 10^{-2}

APPENDIX 2: ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY DATA

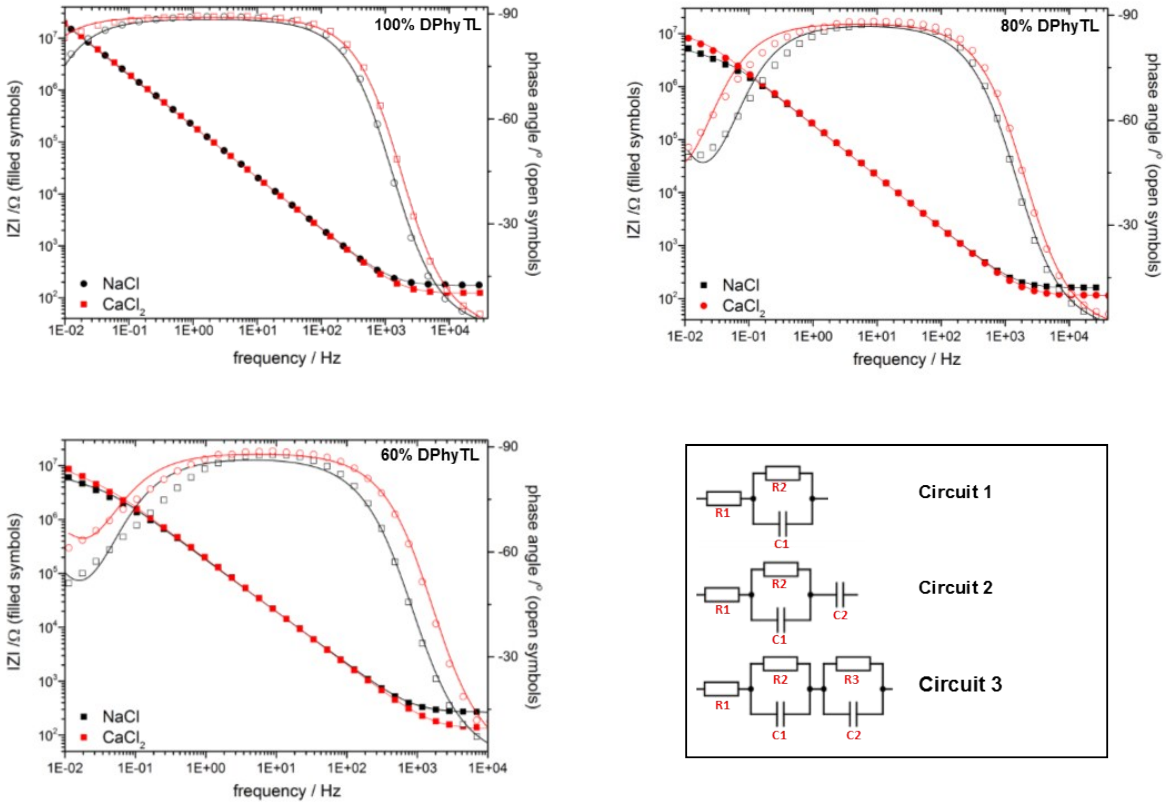

Figure S 8: Bode plots of DPhyPC-tBLMs under NaCl and $CaCl_2$ and schematics of the different circuits used to fit EIS data. R1 is the electrolyte resistance in all circuits, R2 the bilayer resistance and C1 the bilayer capacitance. In circuit 2, C2 represents the capacitance of the gold interface. In circuit 3, the sub-membrane reservoir is populated with ion to an extent that the spacer segment of the bilayer has a resistance and capacitance value (with the gold interface capacitance no longer being visible).

Table S 4: All EIS parameters of Fully tethered LPS-tBLMs of various compositions exposed to EDTA and NaCl electrolytes and Valinomycin. Resistances are given in MΩ.cm^2 and capacitances are given in µF.cm^{-2}

100% RcLPS					
	Bilayer		**Spacer**		**Equivalent Circuit #**
	Resistance	Capacitance	Resistance	Capacitance	
CaCl	26.2 ± 18.4	3.50 ± 0.22			**1**
NaCl	11.6 ± 0.42	3.38 ± 0.03			**1**
30 mM EDTA	7.55 ± 2.14	2.99 ± 0.04			**1**
$CaCl_2$	23.6 ± 1.60	3.08 ± 0.04			**1**
98% RcLPS					
	Resistance	Capacitance	Capacitance		
Bilayer	58.8 ± 4.15	0.79 ± 0.01			**1**
NaCl	34.9 ± 2.29	0.78 ± 0.01			**1**
30 mM EDTA	36.6 ± 5.38	0.73 ± 0.001			**1**
$CaCl_2$	66.3 ± 4.37	0.71 ± 0.01			**1**
94% RcLPS					
	Resistance	Capacitance			
Bilayer	31.0 ± 2.08	1.09 ± 0.02			**1**
NaCl	37.3 ± 2.05	1.03 ± 0.02			**1**
3 mM edta	34.4 ± 1.77	1.00 ± 0.01			**1**
$CaCl_2$	50.6 ± 3.04	0.96 ± 0.01			**1**
100% DPhyPC					
	Resistance	Capacitance			
Bilayer (in $Cacl_2$)	160.3 ± 9.39	0.83 ± 0.01			**1**
NaCl	10.7 ± 3.57	1.28 ± 0.35			**1**
$CaCl_2$	92.3 ± 5.22	0.84 ± 0.01			**1**

Table S 5: All EIS parameters for fully tethered LPS-tBLMs of various compositions after exposure to Colistin sulfate. Resistances are given in MΩ.cm^2 and capacitances are given in µF.cm^{-2}

100% RcLPS			
	Bilayer		**Equivalent Circuit no.**
	Resistance	Capacitance	
Bilayer	138.1 ± 13.5	0.8 ± 0.01	**1**
10 mg/ml Colistin 18 h	196.5 ± 29.4	0.9 ± 0.01	**1**
10 mg/ml Colistin 48 h	119.6 ± 15.3	0.9 ± 0.01	**1**
20 mg/ml Colistin 48 h	108.4 ± 2.3	0.9 ± 0.01	**1**
98% RcLPS			
	Bilayer		
	Resistance	Capacitance	
Bilayer	101.9 ± 11.6	0.8 ± 0.01	**1**
10 mg/ml Colistin 18 h	91.0 ± 19.0	0.8 ± 0.01	**1**
10 mg/ml Colistin 48 h	83.6 ± 18.7	0.8 ± 0.01	**1**
20 mg/ml Colistin 48 h	71.6 ± 18.5	0.8 ± 0.01	**1**
94% RcLPS			
	Bilayer		
	Resistance	Capacitance	
Bilayer	100.6 ± 10.0	0.81 ± 0.01	**1**
10 mg/ml colistin 18 h	73.4 ± 7.41	0.85 ± 0.001	**1**
10 mg/ml colistin 72 h	76.2 ± 1.85	0.93 ± 0.001	**1**

Table S 6: All EIS parameters of sparsely tethered LPS-tBLMs (80% DPhyTL) under EDTA, NaCl and after exposure to valinomycin. Resistances are given in MΩ.cm^2 and capacitances are given in µF.cm^{-2}

100% RcLPS					
	Bilayer		**Spacer**		**Equivalent Circuit no.**
	Resistance	Capacitance	Resistance	Capacitance	
Bilayer	3.52 ± 0.49	1.25 ± 0.09			**1**
NaCl	1.73 ± 0.12	1.25 ± 0.09			**1**
30 mM EDTA	0.001 ± 0.0001	1.24 ± 0.23	0.50 ± 0.02	7.26 ± 0.25	**3**
$CaCl_2$	17.5 ± 0.81	0.77 ± 0.01			**1**
98% RcLPS					
	Bilayer		**Spacer**		
	Resistance	Capacitance	Resistance	Capacitance	**1**
Bilayer	1.75 ± 0.3	1.11 ± 0.6		3.16 ± 0.6	**1**
NaCl	3.54 ± 1.05	1.20 ± 1.05		2.9 ± 1.05	**1**
30 mM Edta	2.61 ± 0.37	0.98 ± 0.06		6.46 ± 2.47	**1**
$CaCl_2$	4.87 ± 0.57	0.86 ± 0.05		11.1 ± 7.81	**1**
94% RcLPS					
	Bilayer		**Spacer**		
	Resistance	Capacitance	Capacitance		
Bilayer	1.24 ± 0.45	1.66 ± 0.23	2.68 ± 0.59		**2**
NaCl	0.22 ± 0.04	1.47 ± 0.13	3.38 ± 0.27		**2**
30 mM edta	1.88 ± 0.61	1.22 ± 0.18	3.24 ± 1.17		**2**
$CaCl_2$	3.37 ± 1.11	1.12 ± 0.19	3.14 ± 1.37		**2**
100% DPhyPC					
	Bilayer		**Spacer**		
	Resistance	Capacitance	Capacitance		
Bilayer (NaCl)	1.79 ± 0.24	4.07 ± 0.34	12.4 ± 3.0		**2**
$CaCl_2$	3.48 ± 0.83	3.73 ± 0.78	15.2 ± 11.8		**2**

Table S 7: All parameters of sparsely tethered LPS-tBLMs after exposure to 10 mg/mL Colistin sulfate. Resistances are given in MΩ.cm^2 and capacitances are given in µF.cm^{-2}

100% RcLPS				
	Bilayer		**Spacer**	**Equivalent Circuit no.**
	Resistance	Capacitance	Capacitance	
Bilayer	5.71 ± 0.0001	1.16 ± 0.67	2.25 ± 2.29	**2**
200 ul Colistin 18	0.01 ± 0.000002	38.2 ± 0.18		**1**
CaCl rinse 18 h	0.04 ± 0.0000009	46.1 ± 0.49		**1**
98% RcLPS				
	Bilayer			
	Resistance	Capacitance		
Bilayer	1.75 ± 0.22	3.14 ± 0.45		**1**
10 mg/mL Colistin 18 h	4.19 ± 1.77	3.02 ± 1.88		**1**
10 mg/mL Colistin 76 h	5.01 ± 0.71	6.49 ± 2.53		**1**
94% RcLPS				
	Bilayer		**Spacer**	
	Resistance	Capacitance	Capacitance	
Bilayer	1.24 ± 0.45	1.66 ± 0.23	2.68 ± 0.59	**2**
10 mg/mL Colistin 18 h	2.26 ± 0.35	20.9 ± 0.45	3.40 ± 0.36	**2**
$CaCl_2$ rinse	0.93 ± 0.04	17.0 ± 0.17		**1**