Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplemental Information
for
Escherichia coli response to subinhibitory concentrations of Colistin: Insights from study of membrane dynamics and morphology.

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February 27, 2022

Supplemental Figures

Supplemental Tables

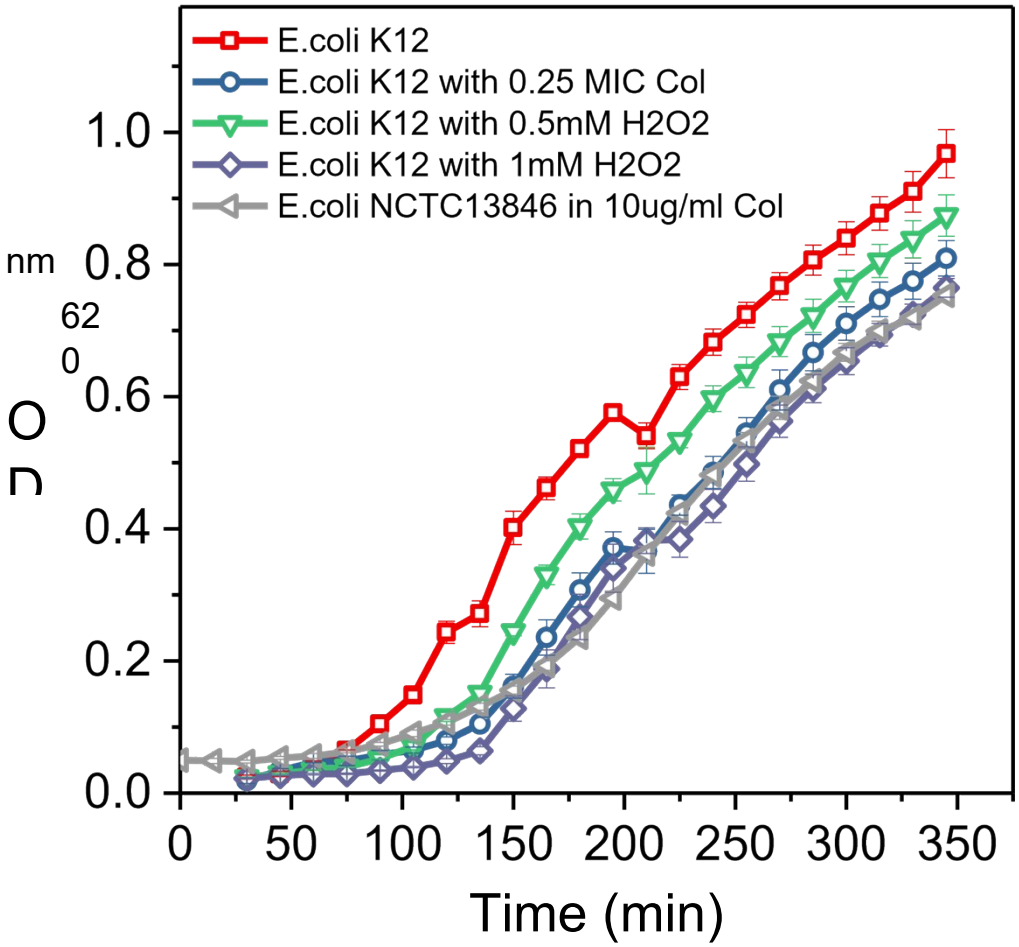

Figure SF1: **Growth curve** of *E. coli*: Absorbance measurements at OD_{620nm} was monitored as a measure of the bacterial cell growth. 1% inoculum of *E. coli* K12 as well as *E. coli* NCTC13846 was added to the growth medium and incubated at 37 °C with continuous shaking. The data was collected at a time interval of 15 min. Growth curves of *E. coli* K12 subjected to colistin treatment (blue circles) as well as hydrogen peroxide treatment (green triangles and purple diamonds) indicate slower growth rate in comparison to the wildtype *E.coli* K12 (red squares). Interestingly, the mutant strain, *E. coli* NCTC13846 (grey triangles) grown in the presence of 10μg/ml colistin also indicated a slower growth rate

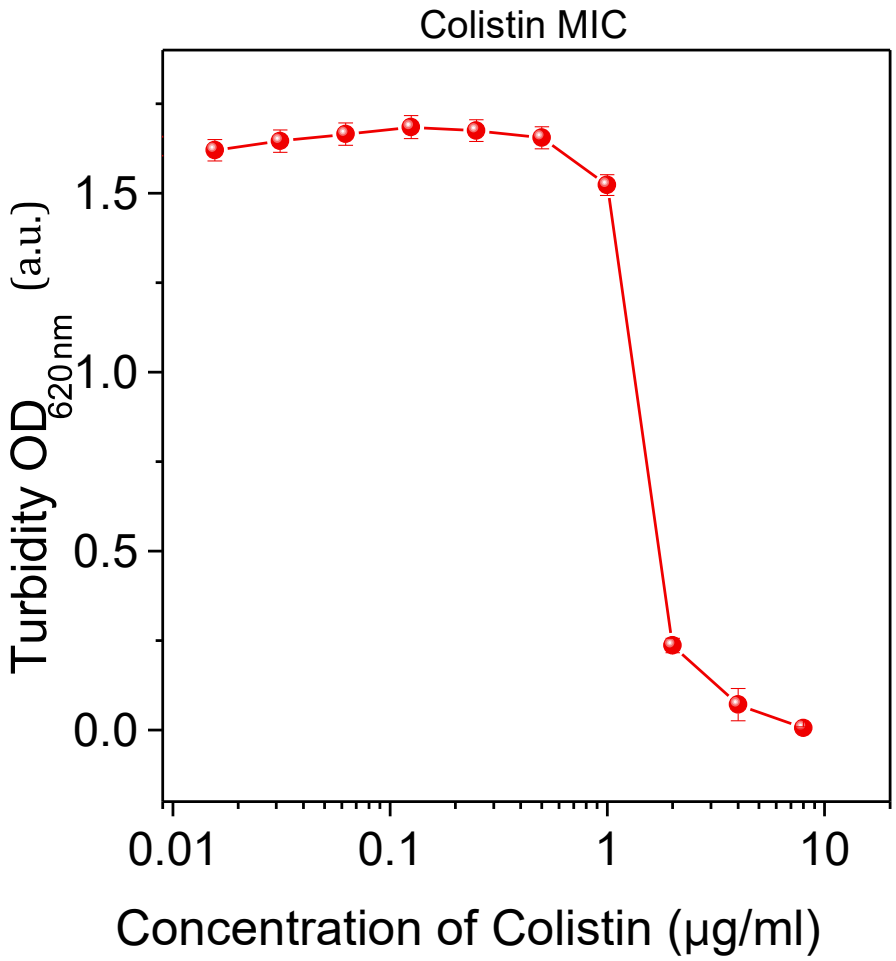

Figure SF2: **Minimum inhibitory concentration of colistin on** *E. coli***:** Absorbance or turbidity measurements at OD_{620nm} was measured as a marker for bacterial cell growth in varying concentrations of colistin to identify the minimum inhibitory concentration (MIC). For calculating the MIC of colistin, 1% primary inoculum of *E. coli* K12 was added to the growth medium with increasing concentration of colistin in a 24-well plate. It was incubated at 37 ˚C overnight (12 - 14 hours) with continuous shaking. The measured values indicate that the *E.coli* K12 strain has a MIC of 2 μg/ml colistin. Based on this measured values, 0.25 MIC, the sub-MIC concentration used in the experiments indicate a colistin concentration of 0.5 μg/ml.

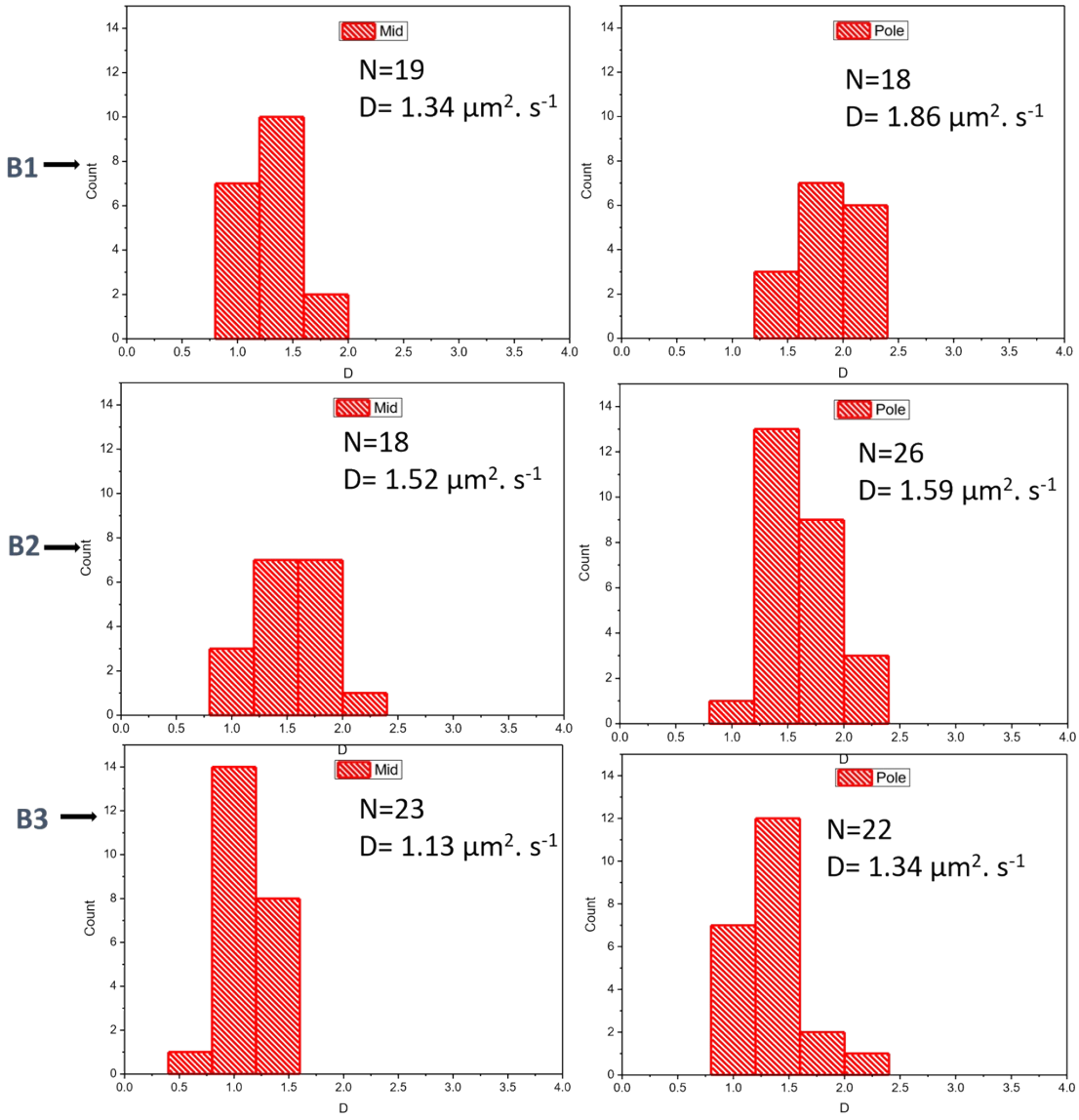

Figure SF3: **Position dependent Nile red diffusion:** The fluorescence correlation spectroscopy (FCS) measurements were carried out on the polar ends and center of the membrane of untreated *E.coli* cells labelled with Nile red. The diffusion coefficients measured for three different bacteria (**B1, B2, B3**) on the center (left panels) and the polar regions (right panels) of the cell envelope are plotted as a histogram. Sample size and the mean diffusion coefficient values are provided inside the plot. Comparing the left and right panels provided we could observe that the diffusion coefficients are not significantly different from each other. Hence, lipid diffusion dynamics is not position dependent. If we compare the B1 data with B2 and B3, We can also interpret that the cell to cell variability in the lipid dynamics is also insignificant. Lipid dynamics is not dependent on individual bacterial cell and is similar in nature across the entire surface of the bacterial membrane.

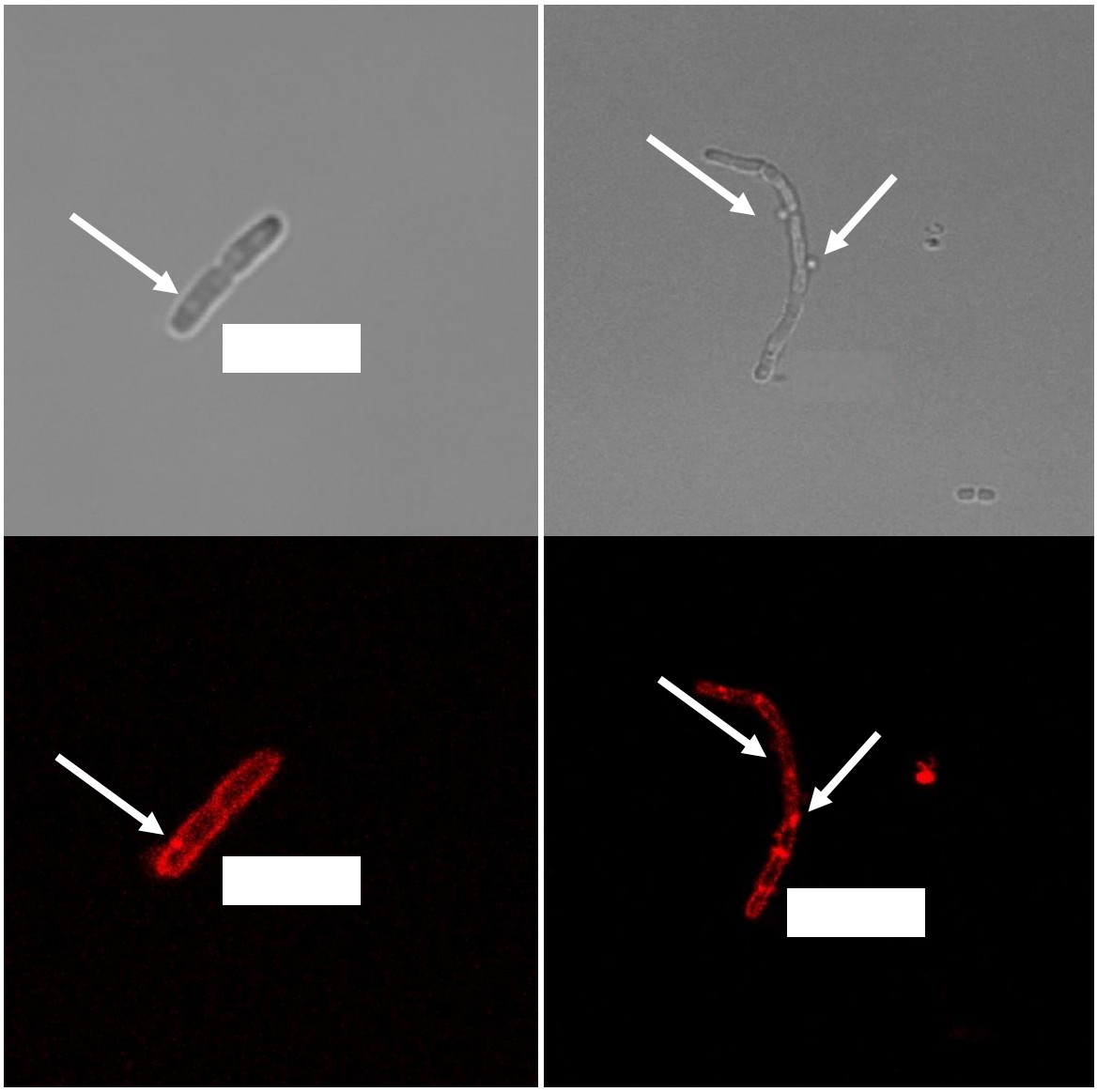

Figure SF4: **Blebs observed in colistin treated** *E. coli***:** Phase contrast and confocal fluorescent imaging of *E.coli* K12 cells labelled with Nile red revealed rod shaped bacterial population of uniform size and shape. When the cells were subjected to 0.25 MIC colistin treatment for 2 hours, morphological changes were observed. Cells were elongated with a significant increase in the average cell length similar to filamentation. Additionally, in few cells, small spherical bleb-like structure was observed as indicated by yellow arrows in the images. These blebs were of different sizes with enhanced Nile red fluorescent intensity. One of the hypothetical reasoning behind the formation of such blebs is that these blebs might have been formed by the bacteria as a defence mechanism by isolating the colistin throgh exocytosis pathway. However, this has to be validated in detailed specific experiments and is beyond the scope of our article. Scale bar is 4 μm.

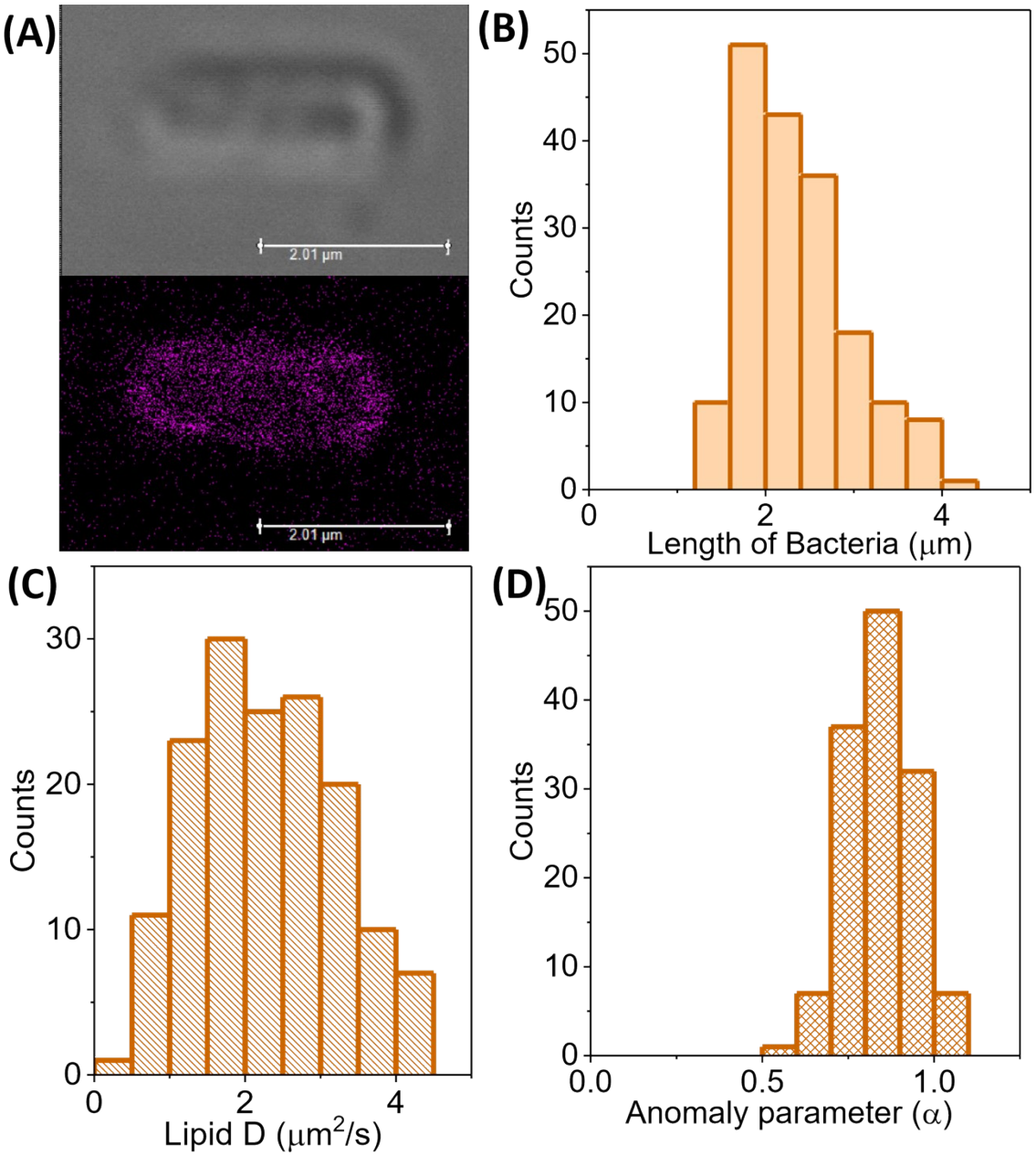

Figure SF5: *E. coli* **treated with 0.125 MIC colistin: A.** Phase contrast and confocal fluorescent imaging of 0.125 MIC treated colistin *E.coli* K12 cells labelled with Nile red. Scale bar is 2 μm. **B.** Size distribution of the bacterial cells treated with 0.125 colistin. Histogram of Nile red diffusion coefficients (panel **C**) and the corresponding anomaly parameter, α (panel **D**) for the 0.125 MIC treated cells.

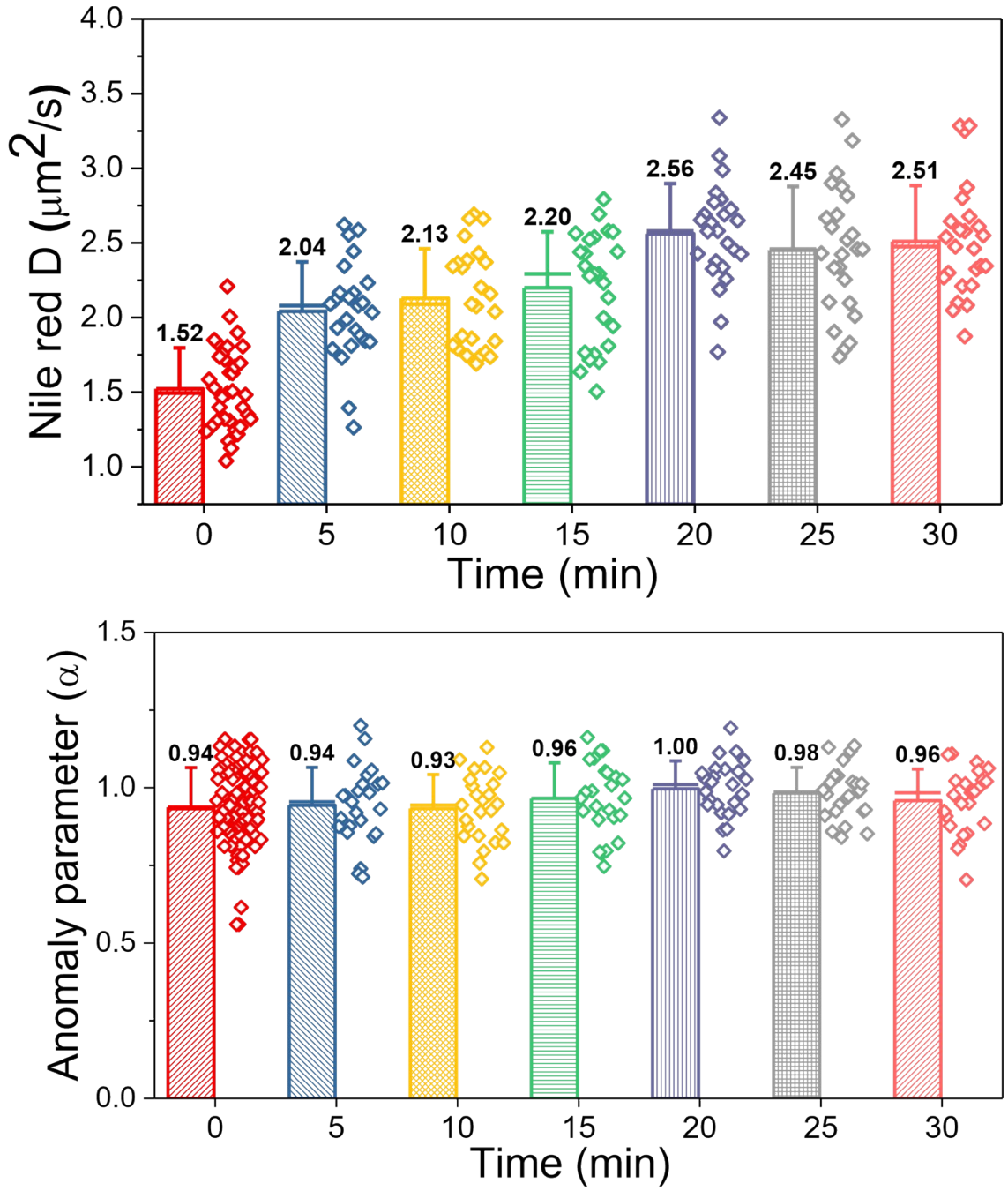

Figure SF6: **Time-dependent diffusion coefficients on colistin treatment:** Inorder to identify the direct interaction of colistin on the cell envelope with respect to altered lipid dynamics, time-dependent FCS was carried out on the cells after addition of 1 MIC (1μg/ml) colistin. The measured values are plotted as a function of time with the mean diffusion coefficient (top panel) and mean anomaly parameters (bottom panel) are labelled on top for each time window. The gradual increase in the diffusion coefficients observed till 20 min indicate that colistin directly interacts with the cell membrane thereby enhancing the lipid dynamics. However, the nature of the diffusion dynamics remain constant as indicated by the anomaly parameter α.

Table ST1: Physical properties of the wild-type and treated *E. coli* cells

Sample	Cell Length μm			Lipid D $\mu m^2/s$			Anomaly parameter α			Roughness R_q nm		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Control	365	2.01	0.51	310	1.43	0.36	310	0.85	0.13	130	4.97	1.89
0.125 MIC colistin	178	2.36	0.63	153	2.29	0.94	153	0.839	0.09	- -	- -	- -
0.25 MIC colistin	200	3.68	1.12	469	2.87	0.86	469	0.94	0.11	109	14.67	4.7
0.5 mM H_2O_2	392	2.88	1.27	382	1.56	0.43	382	0.87	0.13	- -	- -	- -
1 mM H_2O_2	322	3.62	1.42	181	1.55	0.29	181	0.90	0.14	- -	- -	- -
E. coli NCTC 13846	118	2.03	0.36	318	1.63	0.81	318	0.84	0.14	104	6.53	1.84

† N provided in the table represents the number of data points taken for the mean and standard deviation calculation