Electronic Supplementary Material (ESI) for RSC Advances.

SUPPORTING INFORMATION

Insights into Colistin-mediated fluorescence labelling of bacterial LPS

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Contents

1. Absorption and Emission spectra of LPS

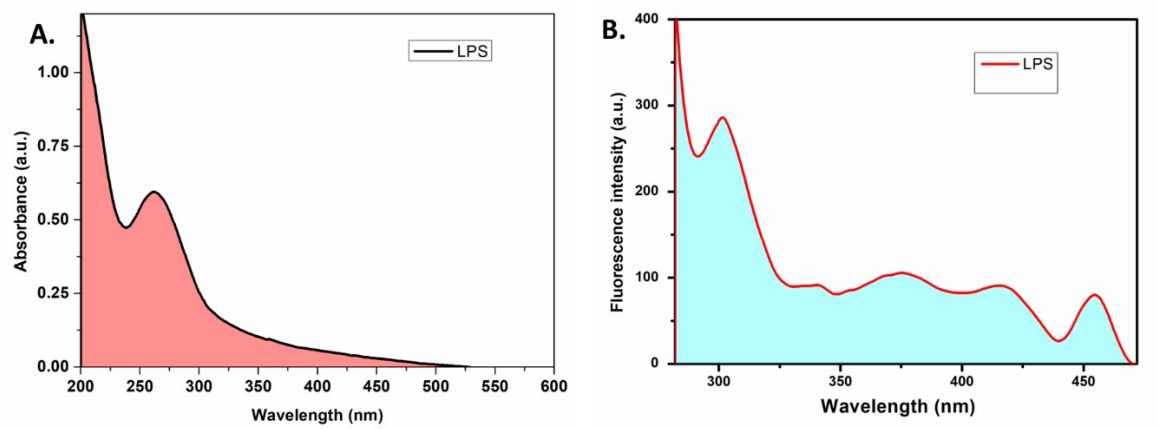

Figure S1. A. Absorption spectra of LPS, B. Emission spectra of LPS upon excitation at 262 nm. LPS (1.718 mg/mL stock solution) has been utilised.

2. UV titration spectra of NAF with Colistin and LPS mixture

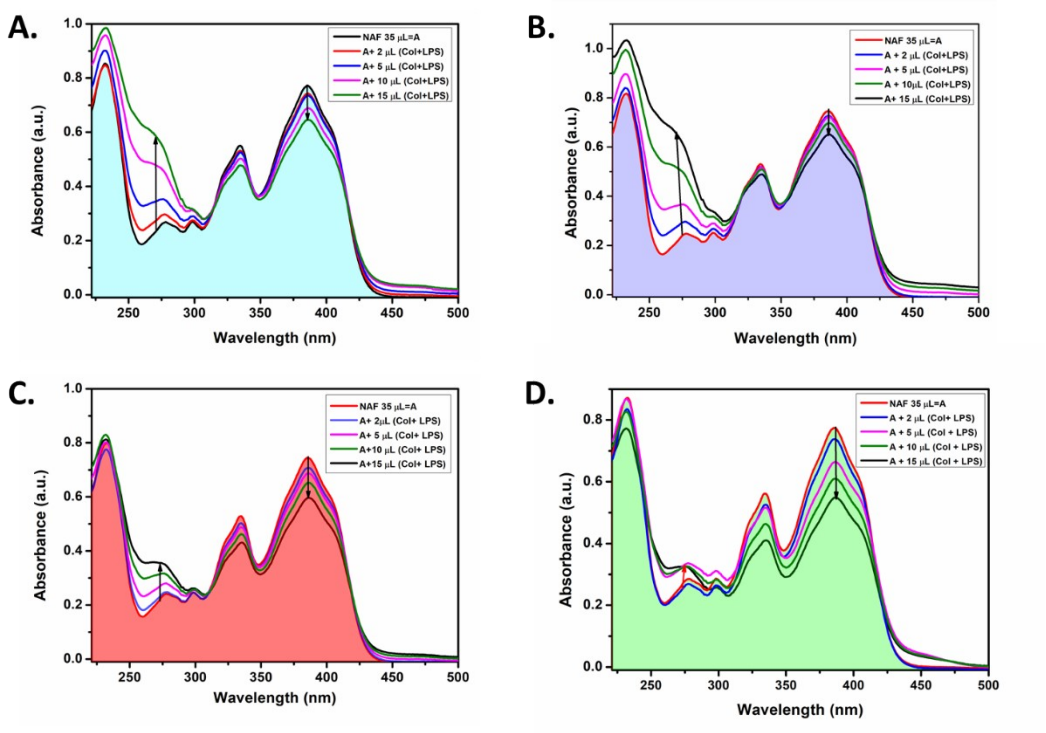

Figure S2. Behavioural change in absorbance bands of NAF upon interaction with colistin-LPS by different ratiometric mixture (v/v) A. colistin: LPS = 1:2, B. colistin: LPS = 1:3, C. colistin: LPS = 2:1, and D. colistin: LPS = 3:1 where NAF (0.017 mg/mL stock solution) , colistin (0.21 mg/ml stock solution) and LPS (1.718 mg/mL stock solution) have been utilised.

3. Fluorescence titration spectra of NAF with Colistin and LPS mixture

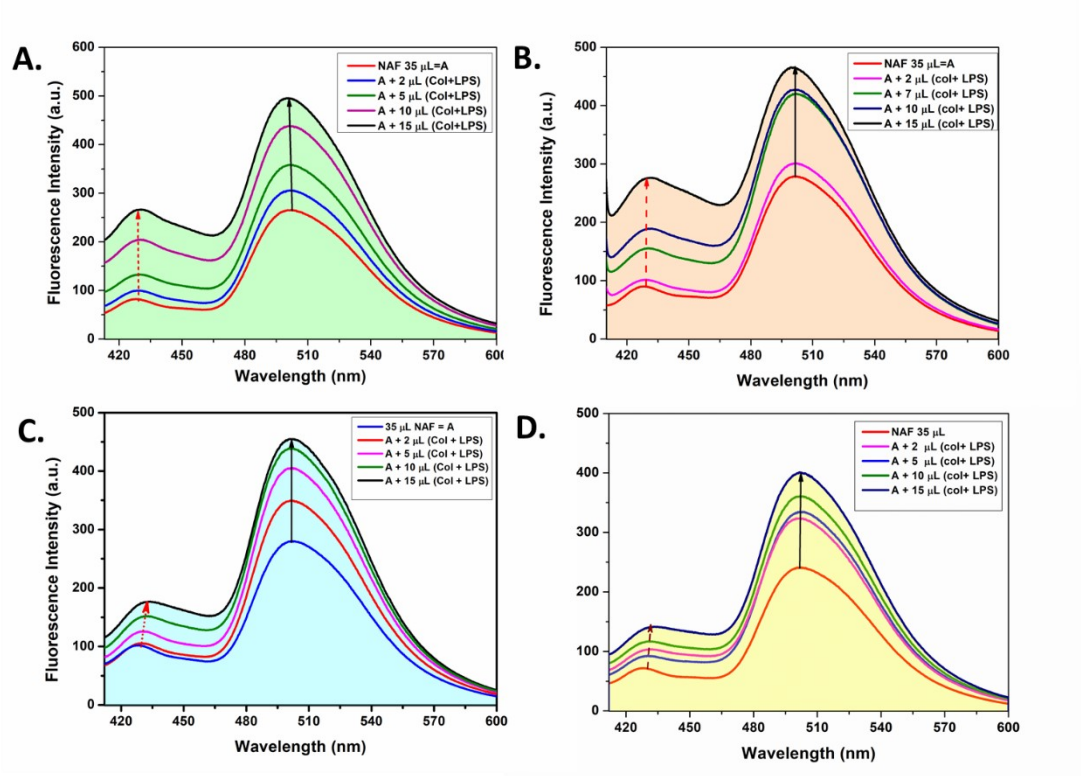

Figure S3. Behavioural change in fluorescence spectra of NAF upon interaction with colistin-LPS by different ratiometric mixture (v/v) A. colistin: LPS = 1:2, B. colistin: LPS = 1:3, C. colistin: LPS = 2:1 and colistin: LPS = 3:1 (λ_{ex}= 384 nm) where NAF (0.017 mg/mL stock solution) , colistin (0.21 mg/ml stock solution) and LPS (1.718 mg/mL stock solution) have been utilised.

4. Quantitative fluorescence response

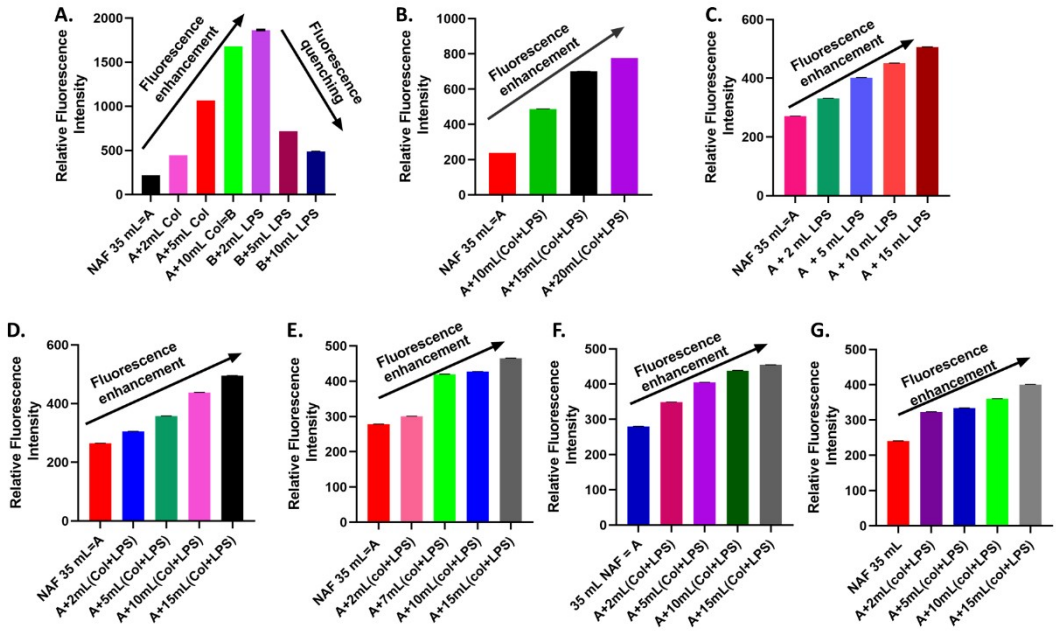

Figure S4. Quantitative fluorescence analysis **A.** NAF (0.017 mg/mL stock) with the sequential addition of colistin (0.21 mg/ml stock) and LPS (1.718 mg/mL), **B.** NAF with simultaneous addition of colistin and LPS (1:1 v/v), **C.** NAF with LPS**,** NAF upon interaction with colistin-LPS by different ratiometric mixture (v/v) D. colistin: LPS = 1:2, **E.** colistin: LPS = 1:3, **F.** colistin: LPS = 2:1, **G.** colistin: LPS = 3:1 (l_{ex}= 384 nm) where NAF (0.017 mg/mL stock solution), colistin (0.21 mg/ml stock solution) and LPS (1.718 mg/mL stock solution) have been utilised.

5. Lipid A domain of LPS Structure

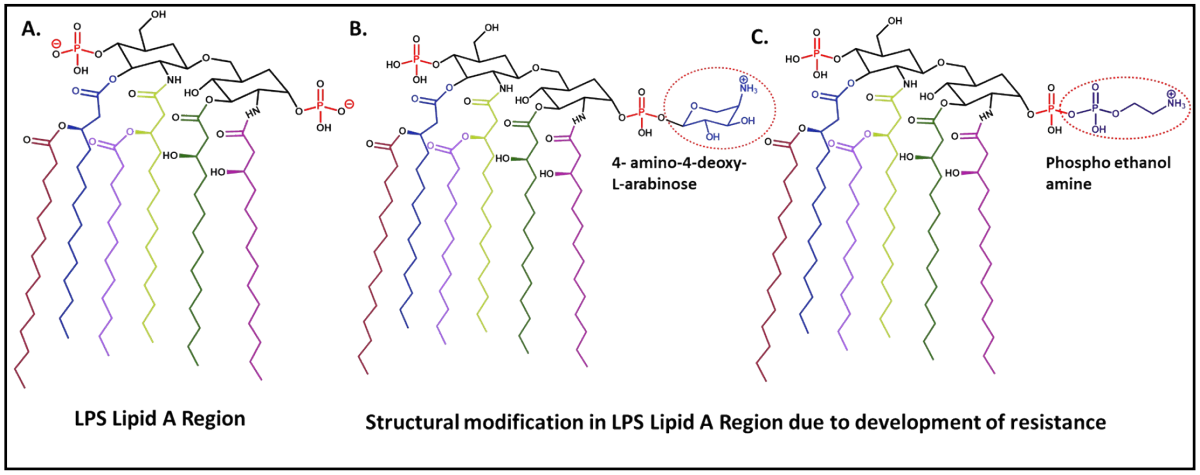

Figure S5. Lipid A region of LPS structure along with their modification in structure due to resistance.

6. Fluorescence Microscopy in Colistin sensitive *E. coli*

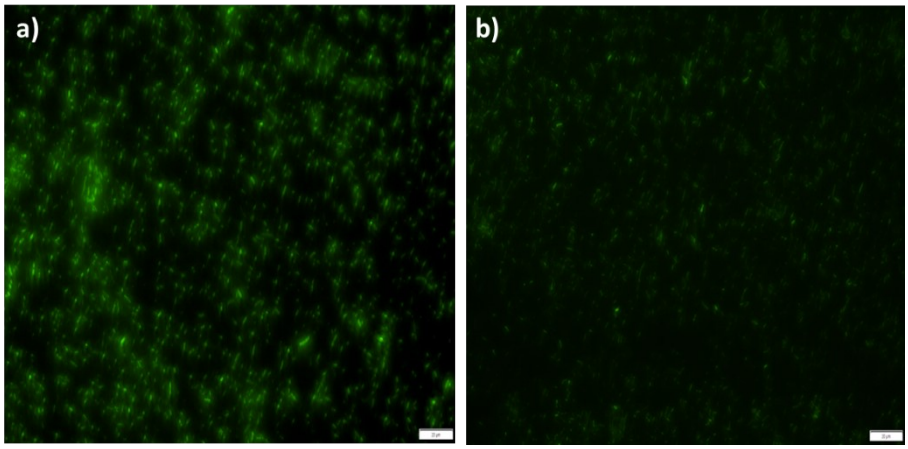

Figure S6. Fluorescence microscopic images of *E.coli* a) FITC filter at 40X. NAF then Colistin b) FITC filter at 40X. Colistin and NAF simultaneous

7. Antibiotic sensitivity Experiment with colistin sensitive *S.typhi*

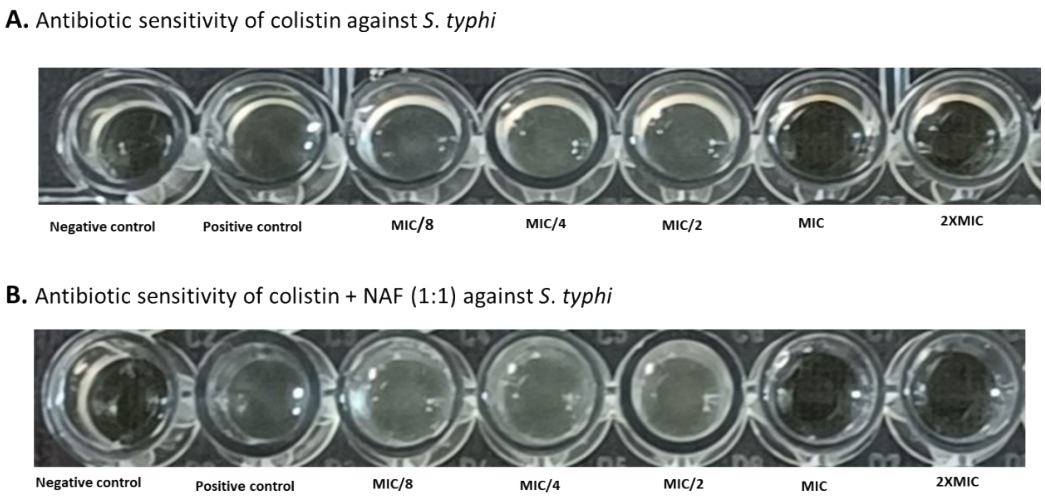

Figure S7. Antibiotic sensitivity well plate experiment

Determination of Minimal Inhibitory Concentration (MIC):

The Minimal Inhibitory Concentration (MIC) was determined in a 96-well microplate using the microdilution method in the Mueller-Hinton broth (Himedia) medium. *Salmonella typhi* ($5x10^5$ CFU/mL) was added to the broth containing different concentrations of colistin and colistin + NAF and then incubated overnight at 37°C. After that, the optical density (OD) was measured using a microplate reader at 600nm. The MIC was determined to be the lowest concentration of antibiotic capable of inhibiting the growth of bacteria.

Table S1: optical density in antibiotic sensitivity assay

	Colistin			Colistin + NAF		
Negative control	0.05	0.05	0.05	0.05	0.05	0.05
Positive control	0.57	0.52	0.49	0.49	0.54	0.49
MIC/8	0.51	0.49	0.57	0.49	0.60	0.49
MIC/4	0.49	0.44	0.46	0.51	0.49	0.50
MIC/2	0.33	0.35	0.39	0.35	0.38	0.39
MIC	0.05	0.05	0.05	0.05	0.05	0.05
2XMIC	0.05	0.05	0.05	0.05	0.05	0.05

Optical density (O.D) at 600nm. The above table shows the optical density in antibiotic sensitivity assay. Colistin and colistin + NAF shows same MIC value against *S. typhi*.

Colistin shows MIC at 4µg/ml, and colistin + NAF(1:1) also shows MIC at 4µg/ml. Thus, from the results of the above experiment, it can be concluded that NAF does not alter the antimicrobial activity of colistin against *S. typhi*.

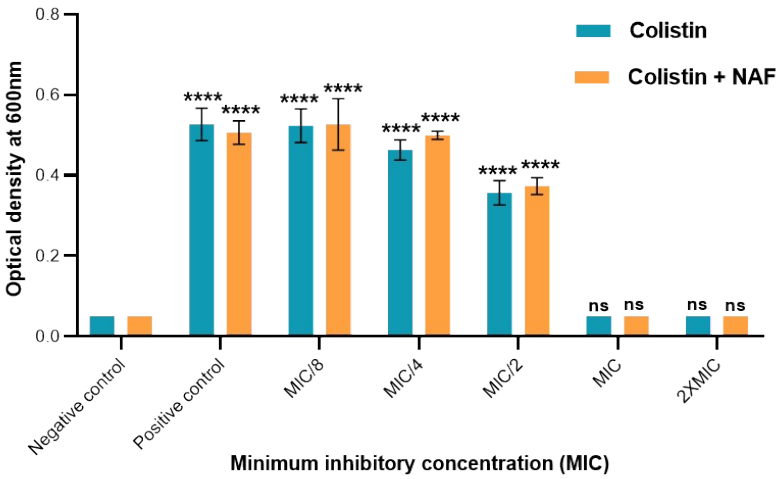

Figure S8. Statistical analysis of optical density data obtained for MIC calculation different *sign [P = <0.0001 (****), P = <0.001 (***), P = <0.01 (**), P =<0.1 (*)] indicates significant difference and ns means not significant by Sidak's multiple comparisons test.

8. Energy minimized LPS 3d Structure

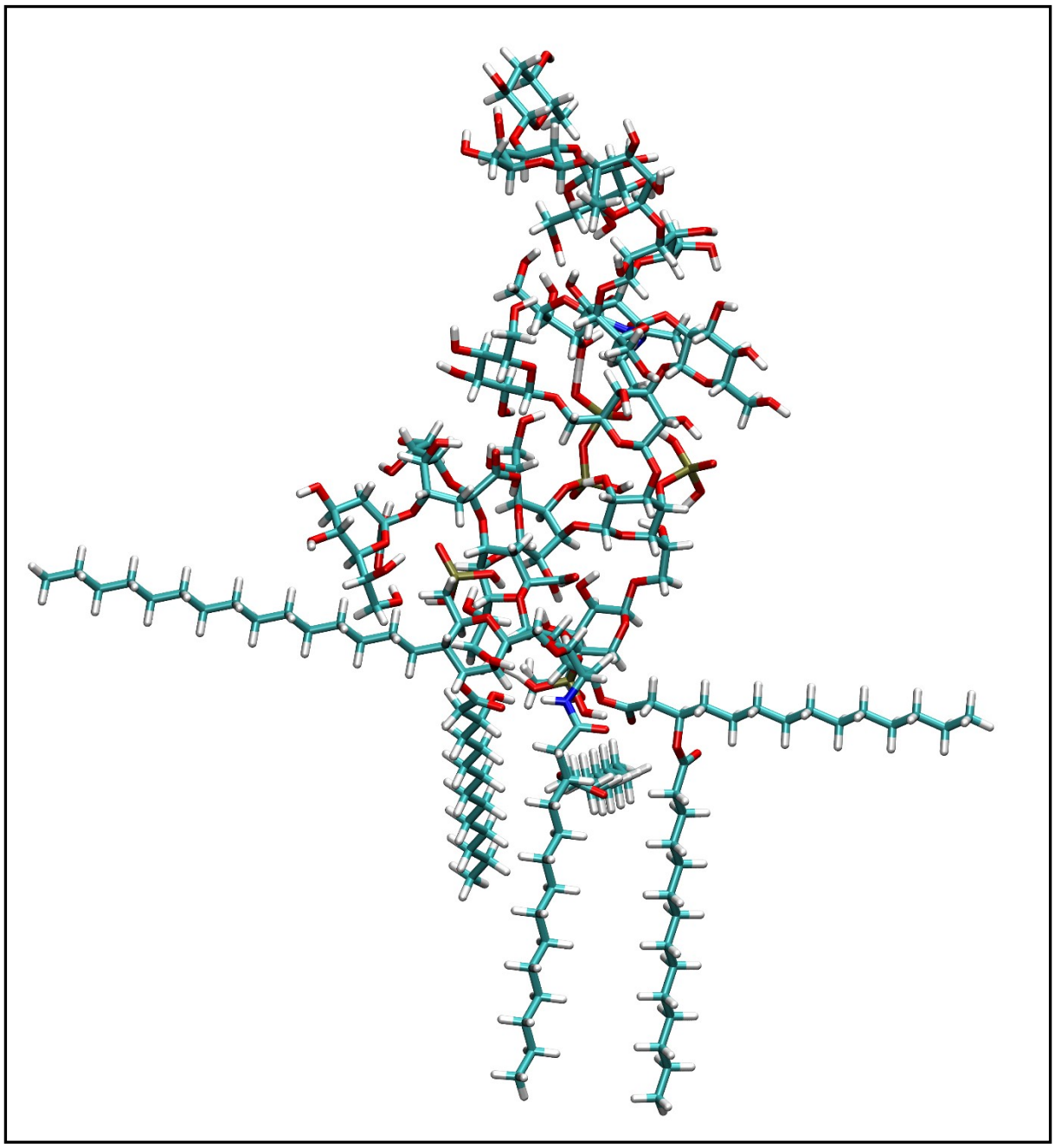

Figure S9. Computationally predicted and simulated the three-dimensional structure of LPS, Optimisation was done by implementing DFT, b3lyp/6-31g level of theory, CPCM solvent model, water as solvent

9. Iso Surface generated from the LPS 3d structure

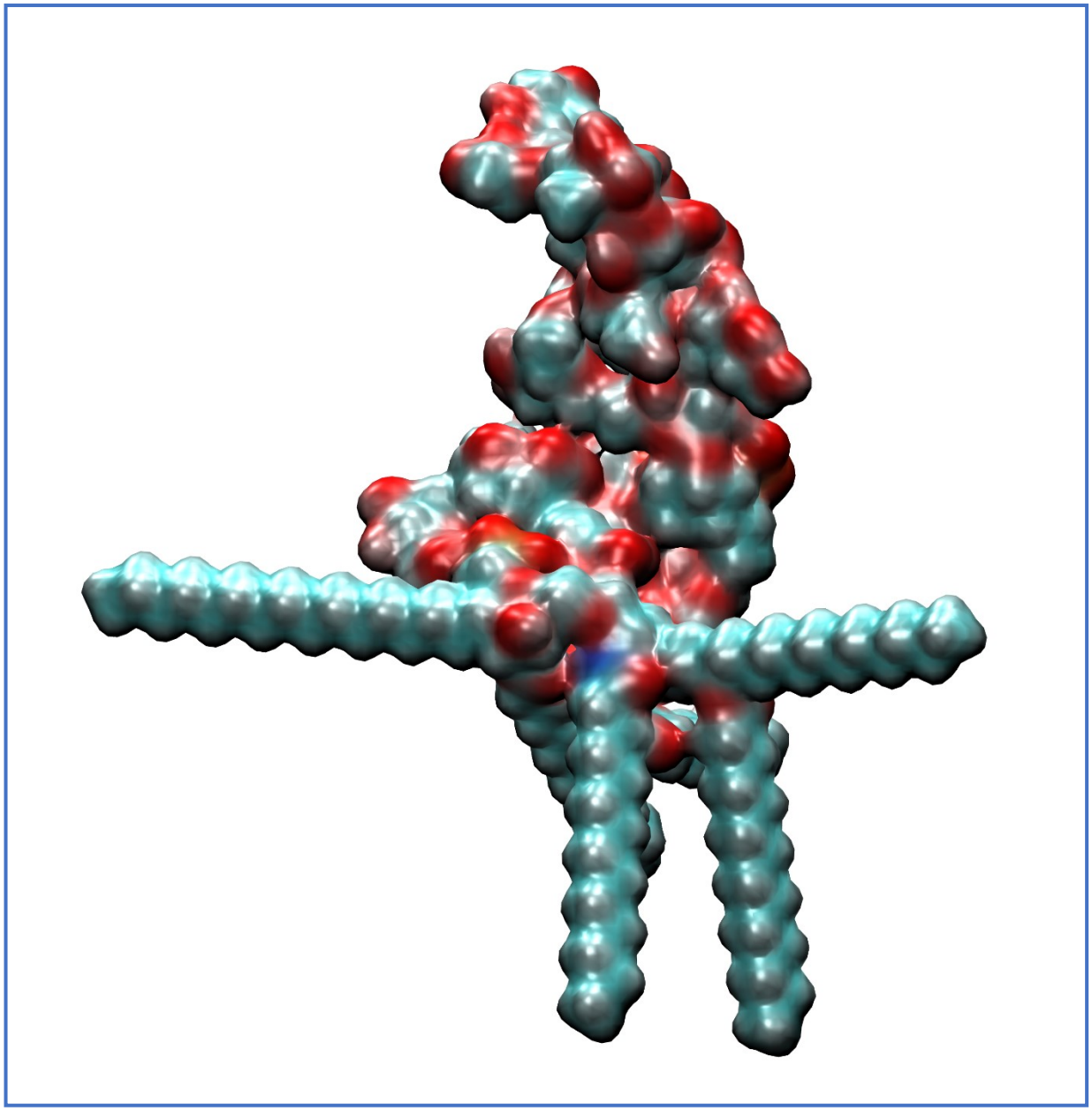

Figure S10. Iso Surface of computationally predicted and simulated structure of LPS.

10. Binding interaction of Colistin with LPS

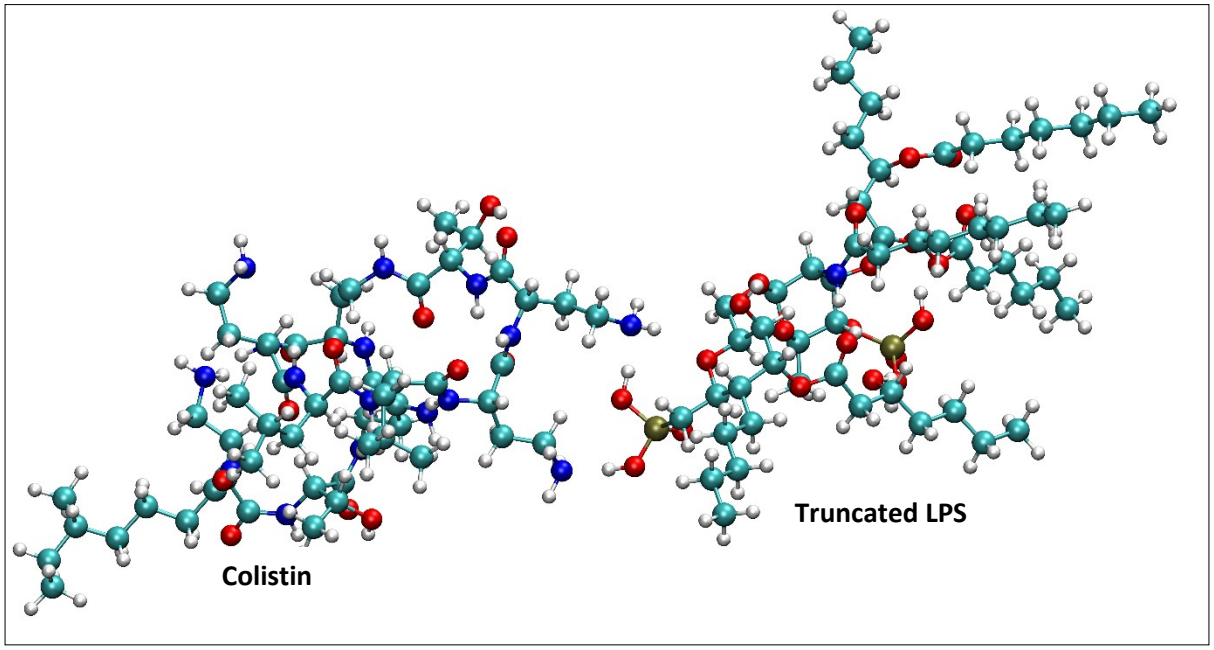

Figure S11. Energy minimised structure of Colistin interacts with truncated of LPS

11. Energy minimisation (MD) and Potential energy diagram

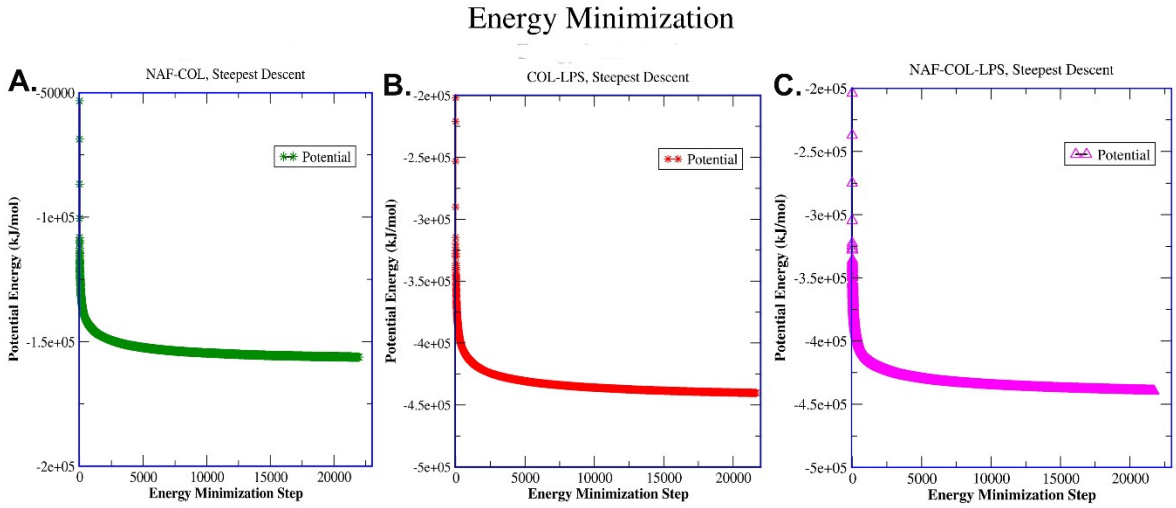

Figure S12. Potential energy diagram of A. NAF-colistin B. colistin-LPS C. NAF-colistin-LPS obtained from MD simulation (energy minimisation)

12. Electrostatic potential (MK charge) and Natural bond orbital calculations

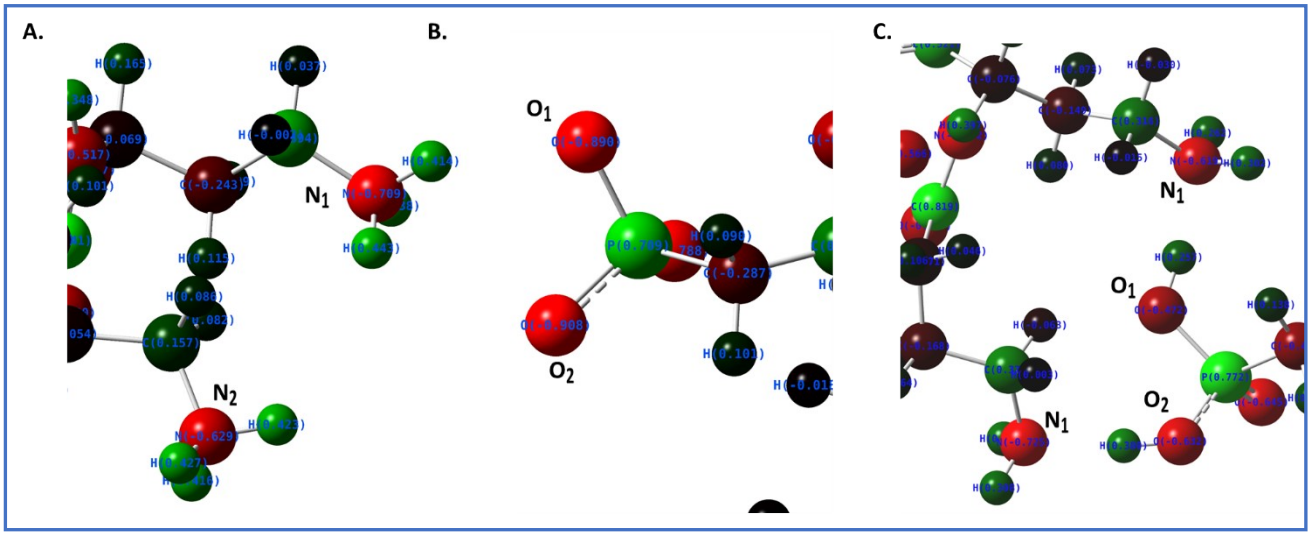

Figure S13. ESP charge analysis (Merz-Kollman charge) of A. Colistin (before interaction with LPS), B. LPS (before interaction with colistin) C. Colistin-LPS conjugate (after interaction)

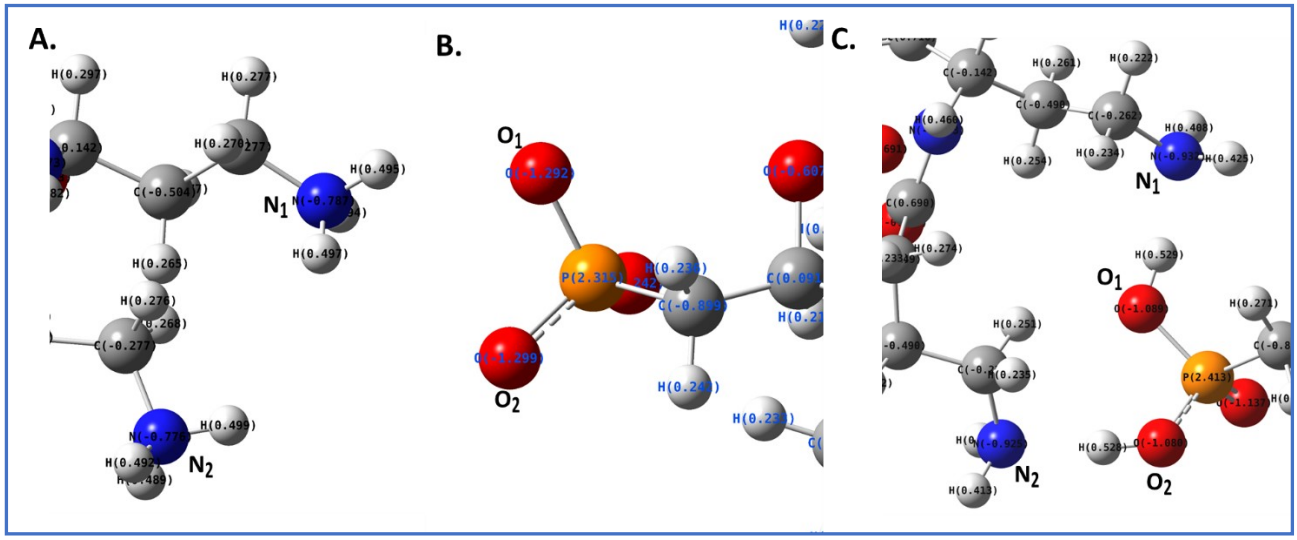

Figure S14. NBO charge analysis of A. Colistin (before interaction with LPS), B. LPS (before interaction with colistin) C. Colistin-LPS conjugate (after interaction)