# Dual-Targeting Sepsis-Associated Inflammation and Infection Using Linoleic Acid-Based Lipid-Polymer Hybrid Nanoparticles

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**Supplementary Materials**

## Materials

Linoleic acid, poly(2-ethyl-2-oxazoline), Acetone, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2′,7′-Dichlorofluorescin Diacetate, acetonitrile, trifluoracetic acid, Muller-Hinton Broth (MHB), Mueller-Hinton Agar (MHA), lipopolysaccharides (LPS) from Escherichia coli (O55:B5), formaldehyde, and paraffin were purchased from Sigma-Aldrich (USA). MitoSOXTM Mitochondrial superoxide indicator, and hematoxylin and eosin (H&E) dye were purchased from Thermo Fisher Scientific (USA). Human cell lines and cell culturing reagents were purchased from Highveld Biologicals (Johannesburg, South Africa) and Whitehead Scientific (Lethabong, South Africa), respectively. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) salt and dimethyl sulfoxide (DMSO) were purchased from Merck Chemicals (Darmstadt, Germany). Sheep blood and heparin-coated microtubes were obtained from United Scientific (South Africa). Methicillin-resistant Staphylococcus aureus (MRSA Rosenbach ATCC BAA 700699) and Staphylococcus aureus (ATCC 25923) were purchased from DLD Scientific (South Africa). Microscale thermo-phoresis (MST) reagents and consumables were ordered from Nano-Temper Technologies (Munich, Germany). ADAM10 protein, alpha-hemolysin, FITC Annexin V Apoptosis Kit with PI, Human IL-1β ( Interleukin-1Beta) ELISA kit, human TNF-α (Tumor Necrosis Factor Alpha) ELISA kit, LEGENDplexTM Multi-Analyte Flow Assay kit were purchased from Biocom Africa. Purified distilled water (D.W) was obtained from a mill-Q water purification system (Millipore Corp., USA). All reagents and solvents employed in the present study were of analytical grade.

**Table S1**. Size, PDI, and ZP for different surfactants used in the formulation of VCM-LIN-P2O NPs. Results represented as mean ± SD (n = 3).

| Surfactant type | Size | PDI | zeta |
|---|---|---|---|
| Span 20 | 288.9 +/- 59.21 | 0.354 +/- 0.071 | -4.68 +/- 0.560 |
| Poloxamer 188 | 216.4 +/- 2.831 | 0.239 +/- 0.020 | -8.12 +/- 0.425 |
| Tween 20 | 148.9 +/- 0.8963 | 0.225 +/- 0.003 | -36.1 +/- 4.34 |
| Tween 80 | 165.9 +/- 4.285 | 0.257 +/- 0.008 | -29.7 +/- 1.16 |
| Poloxamer 407 | 157.6 +/- 1.332 | 0.213 +/- 0.008 | -18.0 +/- 1.01 |

**Table S2.** Kinetics of drug release of VCM-LIN-P2O NPs under physiological condition (pH 7.4) using various mathematical models.

| Model | Equation | $R^2$ | RMSE | Release exponent *n, β* |
|---|---|---|---|---|
| Zero Order | Q=k*t | -0.0729 | 25.8788 | |
| First Order | $Q = Q_0 . e^{kt}$ | 0.9447 | 5.8755 | |
| Higuchi | $Q = k . t^{½}$ | 0.9354 | 6.3490 | |
| Korsmeyer-Peppas | $Q = k . t^n$ | 0.9763 | 3.842 | 0.408 |
| Hixson-Crowell | $Q^{⅓} = kt + Q_0^{⅓}$ | 0.8973 | 8.0050 | |
| Weibull | $Q = 1 \exp [-(t)^{a/b}]$ | 0.9990 | 0.7770 | 0.814 |

$R^2$ = linear regression coefficient, RMSE = Root mean square error

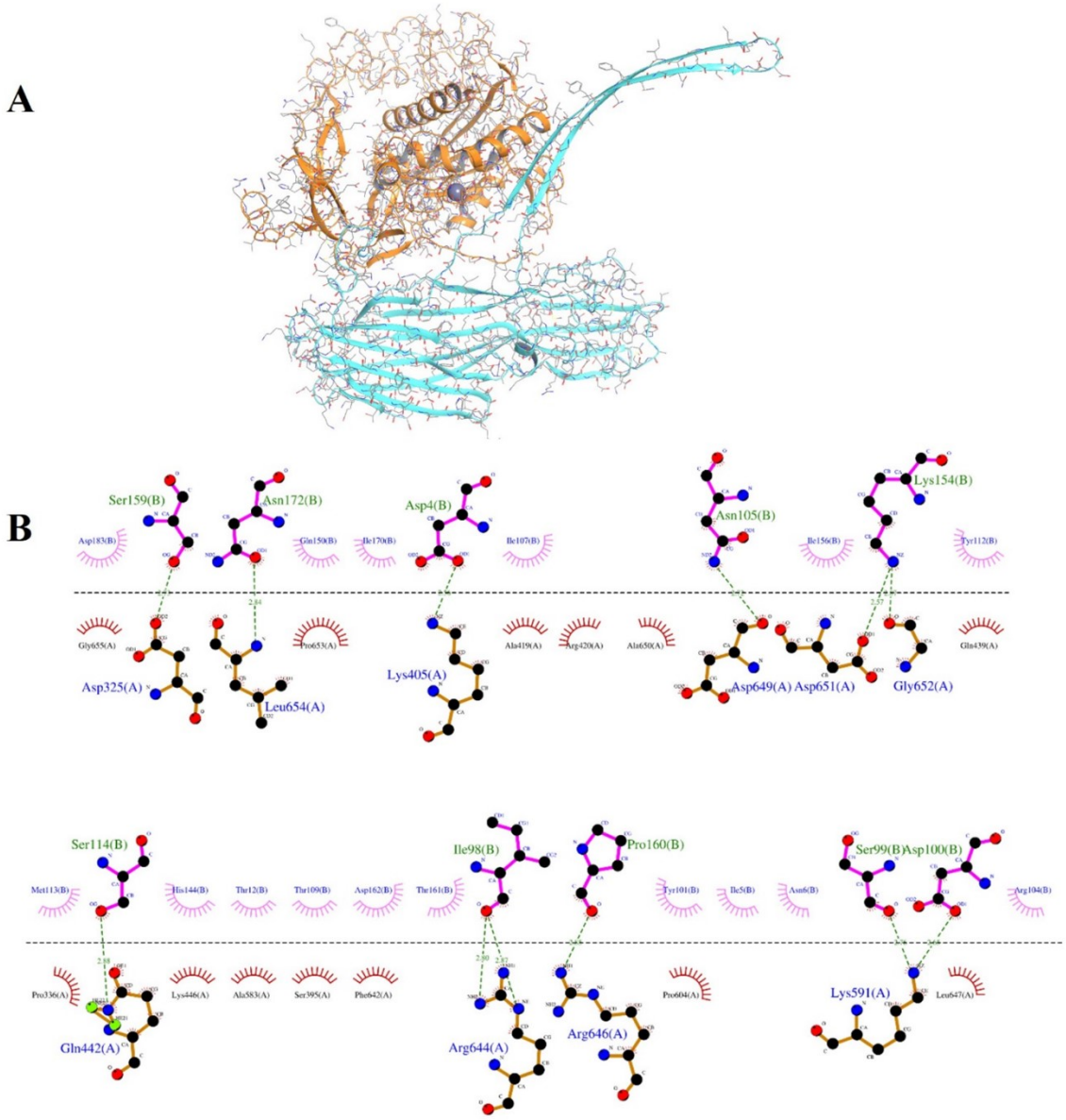


**Fig. S1.** **(A)** Docked poses of ADAM10 and alpha hemolysin (α-H) and **(B)** 2D representation of the ADAM10-3ANZ protein-protein interface. Residues from ADAM10 (chain A) are shown in blue, while interacting residues from 3ANZ (chain B) appear in green. The dotted black line marks the interface boundary between the two proteins. Hydrogen bonds and hydrophobic interactions are illustrated as green dashed lines and red arcs, respectively.

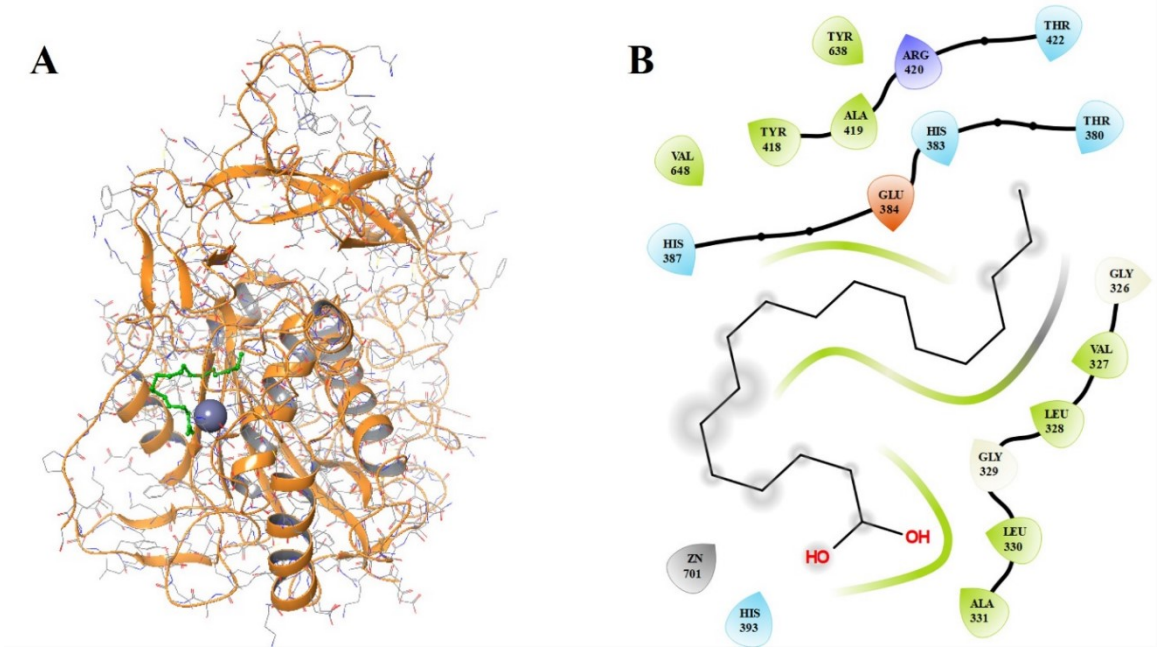


**Fig S2.** Docking poses and binding interactions of ADAM10 and Linoleic acid (Green).

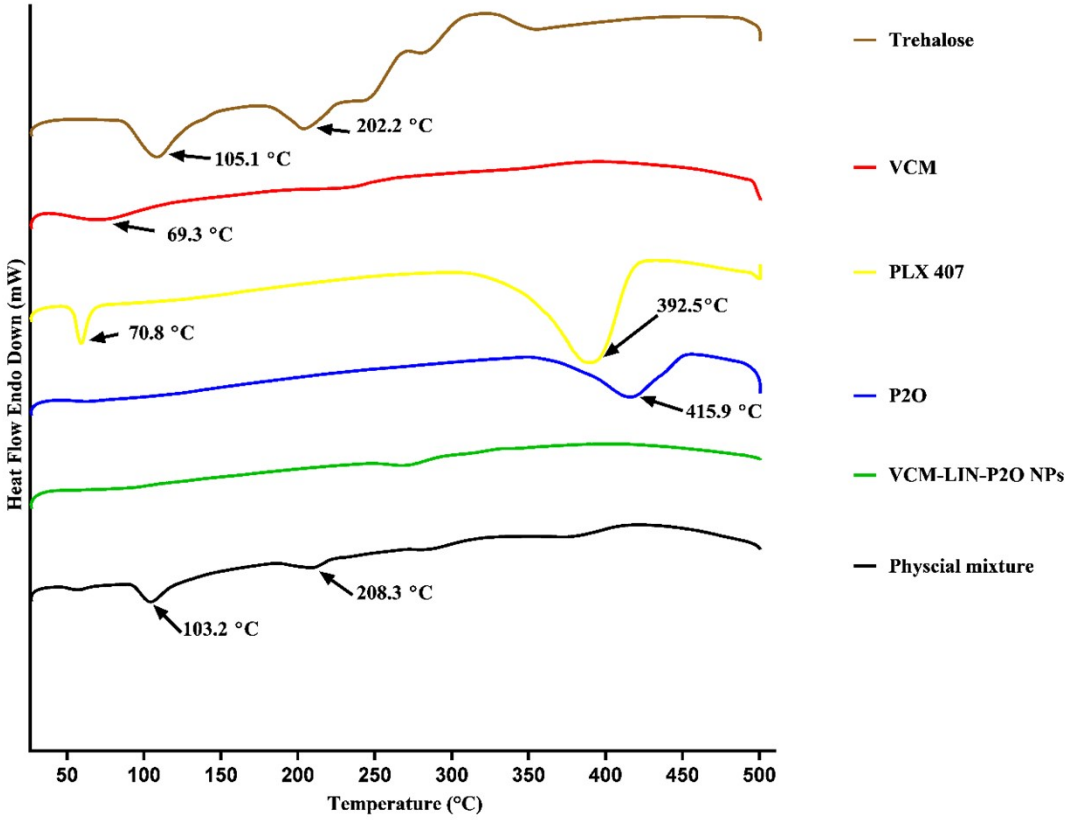


**Fig. S3.** DSC thermogram of trehalose, vancomycin (VCM), poloxamer 407 (PLX407), poly(2-ethyl-2-oxazoline) (P2O), VCM-LIN-P2O NPs, and the physical mixture, which confirms the successful encapsulation of VCM in the VCM-LIN-P2O NPs.

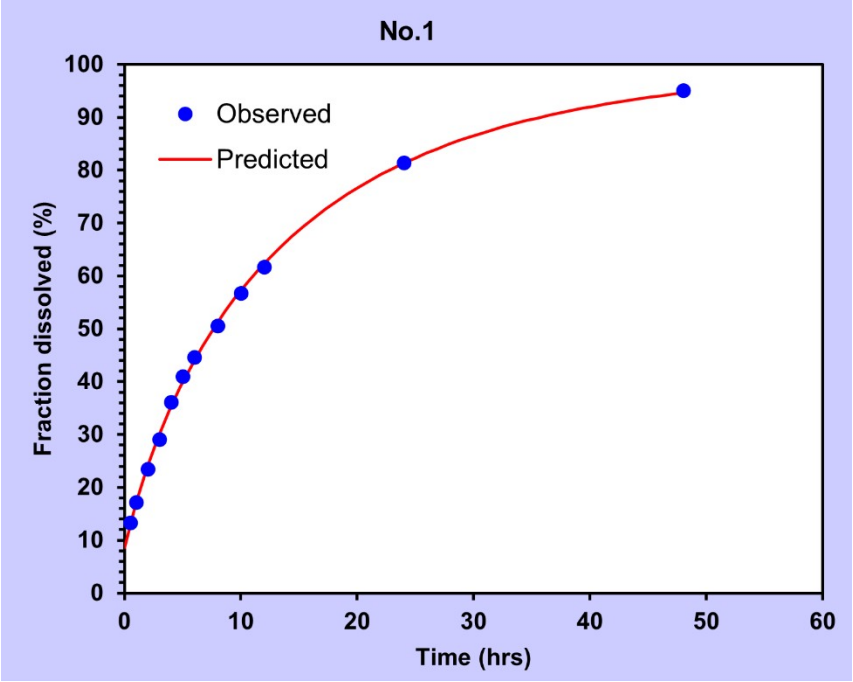


**Fig. S4**. Weibull fitting of VCM-LIN-P2O NPs *in vitro* drug release profile.

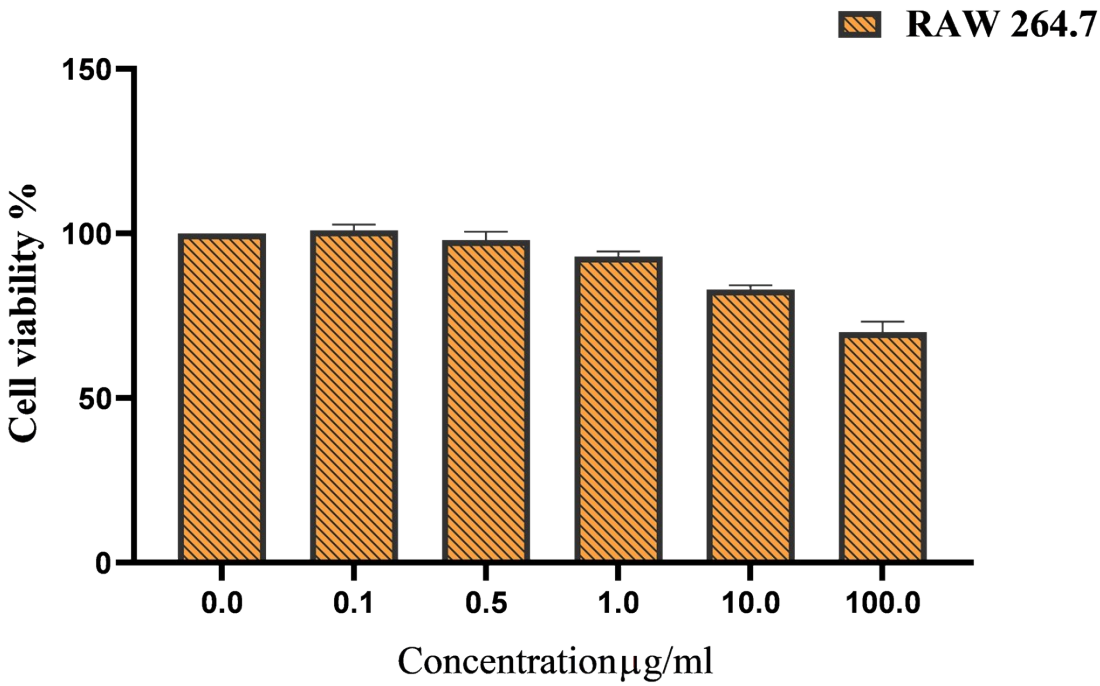


**Fig. S5.** Percentage of RAW264.7 cell viability after exposure to different LPS concentrations compared to untreated controls.