Supplementary information

Site specific immobilization of a potent antimicrobial peptide onto silicone catheters: evaluation against urinary tract infection pathogens

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Running title: Antimicrobial characterization of covalently immobilized CysLasio-III.

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Materials and methods

Peptide activity and selectivity determination

Minimal inhibitory concentration (MIC) of Lasio-III variants was determined by the micro-broth dilution method.[1] Briefly, overnight cultures of bacteria in Luria-bertani (LB) were sub-cultured to mid-log phase and diluted to a final concentration of 10^6 CFU/ml. 50 µl of that bacteria culture was added to 50 µl of serially diluted peptide solutions to achieve final peptide concentrations ranging from 0.4 to 28 µM. The plates were incubated at 37 ^{0}C overnight. Wells without peptide served as the positive control while uninoculated media served as the negative control. MIC was taken as the lowest peptide concentration that completely inhibited bacteria growth. Experiments were conducted in triplicates and the mean values were reported. Minimal haemolysis concentration (MHC_{50}) of CysLasio-III was calculated using freshly collected human Red Blood Cells (hRBC) based on an established protocol.[2] The ratio of the MHC_{50} and MIC values was taken as the cell selectivity index (CI) of the peptide.[3, 4]

NPN Uptake assay

E. coli cells grown to mid-log phase were centrifuged at 3000 x g and resuspended in 10 mM phosphate buffer (pH 7.2) to obtain a final OD_{600} of 0.6. Stock solution of NPN in acetone was added to 500 µl *E. coli* cells containing varying peptide concentration (1-15 µM), to obtain a final concentration of 10 µM of NPN. The NPN dye was excited at 350 nm and the increase in fluorescence intensity from emission maxima at 410 nm was recorded using a fluorescence spectrophotometer (LS5, Perkin Elmer, U.S.A.). The basal fluorescence value (i.e. fluorescence of NPN in 500 µl *E. coli* cells) was subtracted from fluorescent readings of all peptide-containing samples. The maximum value of NPN uptake was determined by treating the cells with 10 µl polymyxin B sulfate (from 0.64 µg/ml stock solution), which is an efficient outer

membrane permeabilizing agent.[5] The outer membrane permeabilization activity of Lasio-III variants was assessed by comparing fluorescence readings of peptide-treated cells relative to polymyxin B-treated cells.

Isothermal titration calorimetry (ITC)

All ITC experiments were performed on a VP-ITC calorimeter (Micro Cal Inc.) at 30˚C with DPC lipid micelles. For titration with DPC micelles, peptide and lipid were both dissolved in 10 mM phosphate buffer (pH 7.2). Twenty injections of 5 µl aliquots of the DPC micelles (100 mM) were added to the sample cell containing the peptides at 5 µM, while the reference cell contained 10 mM phosphate buffer. Each titration was performed at an interval of 300 s, stirring speed of 300 rpm and the heat exchange was recorded. In all cases, the heats of dilution of the micelle alone into the buffer were subtracted from the titration data. The resulting data were integrated with Micro Cal Origin 5.0, fitted with models provided in the software and analyzed to determine the association constant, K_a, and the enthalpy change, ΔH. The Gibb's free energy change, ΔG, and entropy change, ΔS, were calculated from the fundamental thermodynamic equations: $\Delta G = -RT \ln K_a$ and $\Delta S = (\Delta H - \Delta G)/T$, respectively.

Results

TABLES

Table S1 Average helical contents of Lasio-III and CysLasio-III.

Solvent	**% helicity (190-260nm)**	
	Lasio-III	**CysLasio-III**
Water	16.6	17.6
10% TFE	18.5	23.7
20% TFE	46.3	62.6
40% TFE	60.1	72.5

Table S2 Antimicrobial activity of immobilized CysLasio-III against bacteria.

Bacteria	**Colony count**[a]	**Inhibition (%)**
E. coli	0	99.9
E. faecalis	0	99.9

[a]CFU counts following modified ISO protocol. For details, please see materials and methods section.

FIGURES

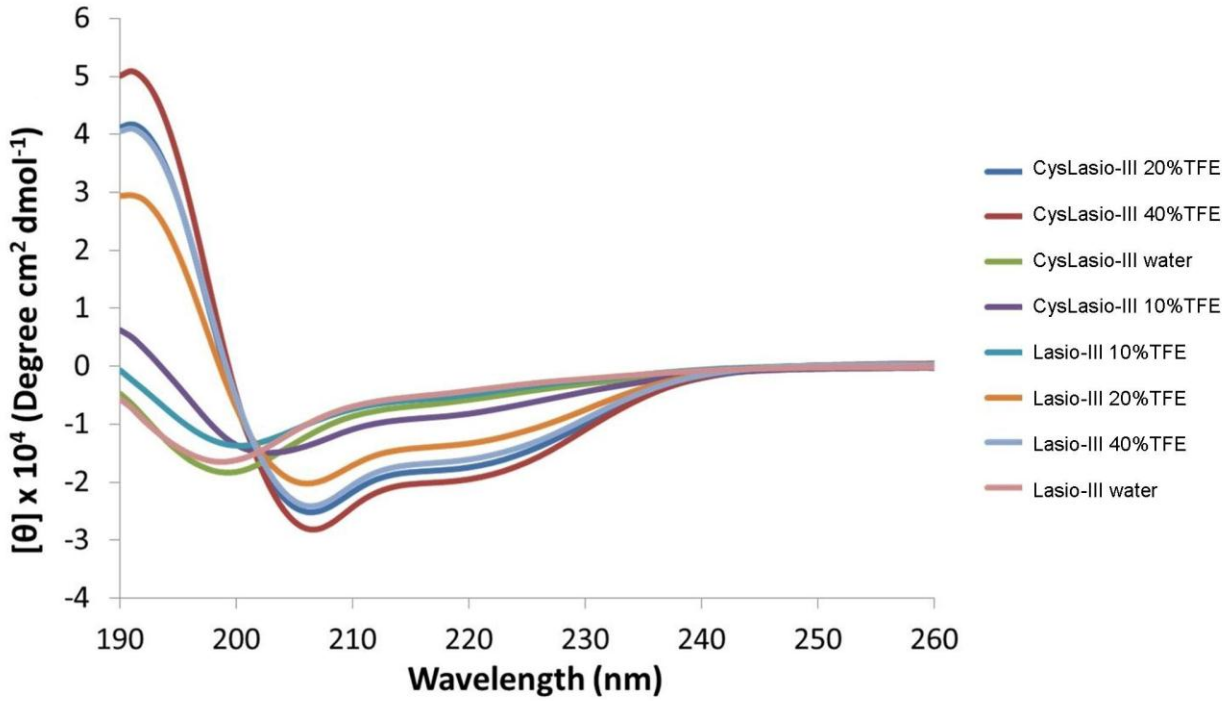

Fig. S1 CD spectra of peptides Lasio-III and CysLasio-III with 10, 20 and 40% TFE and water (vol/vol). Peptide concentration was 0.25 mg/mL, θ is the mean molar ellipticity (Degree cm^2 $dmol^{-1}$).

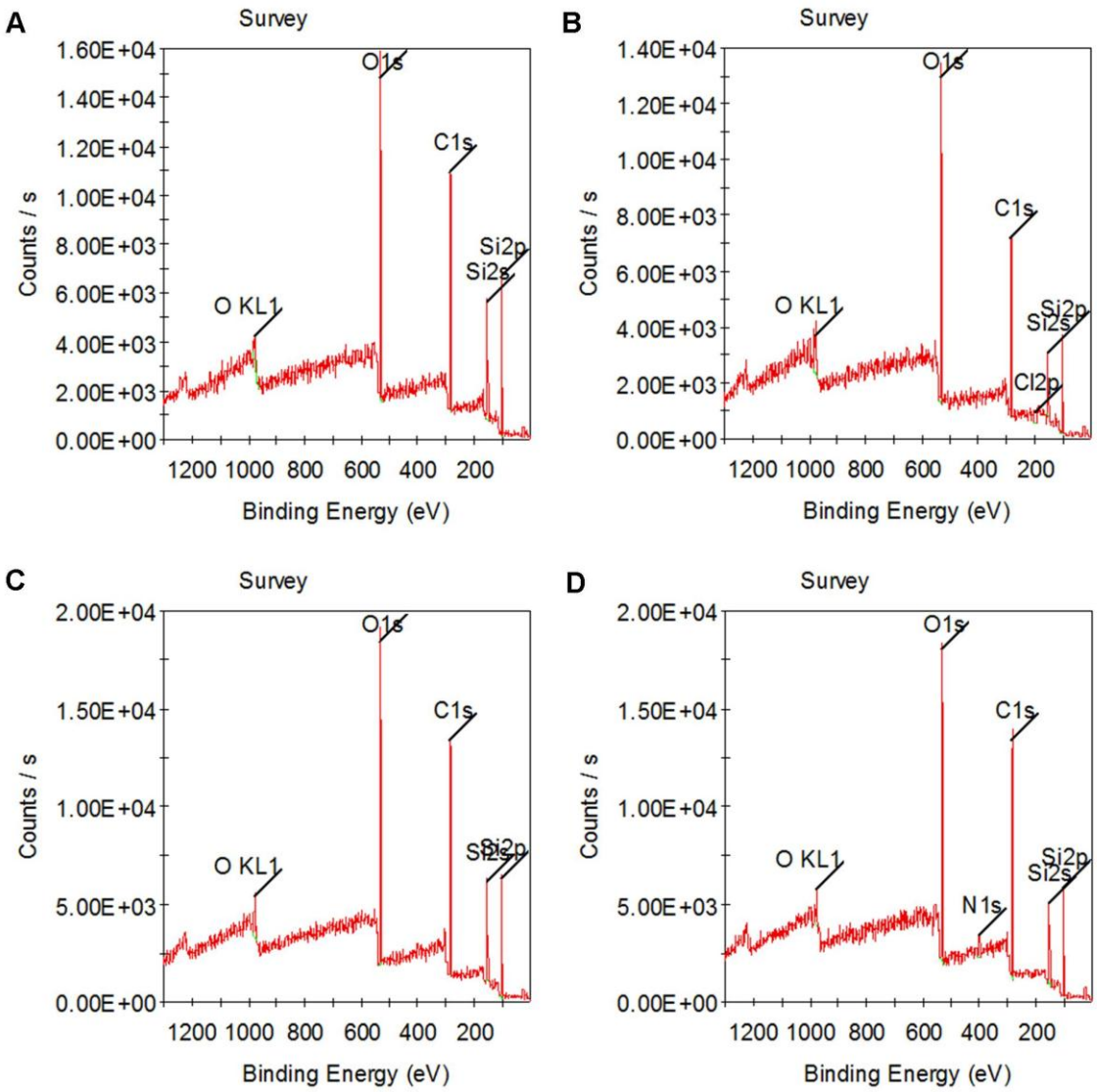

Fig. S2 Characterization of Catheter-CysLasio-III. Wide scan spectra of obtained from XPS analysis. Catheter-control (A), Catheter–amine (B), Catheter–PEG_{12}-Mal (C), and Catheter–CysLasio-III (D).

References

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