SUPPLEMENTARY MATERIALS

Comparison of the activity of two elastin-like recombinant carriers fused to the antimicrobial peptide indolicidin

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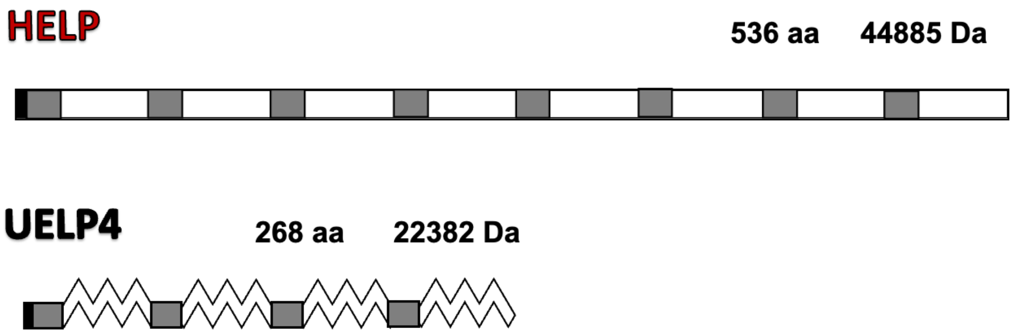
A



B

<table>
<tr><td>biopolymer</td><td>Domain</td><td>aa sequence</td></tr>
<tr><td>HELP

UELP4</td><td>Cross-linking</td><td>AAAAAAKAAAKAAQF
| | | | | | | | | | | | | | |
AAAAAAKAAAKAAQF</td></tr>
<tr><td>biopolymer</td><td>Domain</td><td>aa sequence</td></tr>
<tr><td>HELP

UELP4</td><td>Elastin-like</td><td>GLVPGVGVAPGVGVAPGVGVAPGVGLAPGVGVAPGVGVAPGVGVAPGIAP
|| || || || || || || ||
GLGAGVPGLGVGAGVPGFGVGAGVPGLGVGAGVPGFGVGAGVPGLGAP</td></tr>
</table>

C ILPWKWPWWPWRR 13 aa 1907 Da

Fig 1S. The two elastin-like biopolymers that were used as carriers in this study. (A) Scheme of the primary structure of the two biopolymers: grey, cross-linking domains; white, elastin-like sequences. (B) The amino acid sequence of the crosslinking (grey) and of the hydrophobic elastin-like domains (white) that compose the two biopolymers. The hexapeptidic repeats that characterize the HELP sequence are boxed in red, and the nonapeptidic repeats that characterize UELP4 are boxed in black. The canonical pentapeptidic motifs are in bold. (C) The amino acid sequence of indolicidin.

Calculation of the accessible surface area of indolicidin. The PDB file of the 3D structure of indolicidin from the PDB entry 5zvf was used to estimate the solvent-accessible surface area using the online software GETAREA (https://curie.utmb.edu/getarea.html) [1].

Table 1S. Theoretical solvent accessibility surface area of the indolicidin.

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POLAR area/energy          =         566.48
APOLAR area/energy         =        1808.34
UNKNOW area/energy         =           0.00
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Total area/energy          =        2374.82
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Differential Scanning Calorimetry. The thermal properties of the proteins in solution were evaluated by Differential Scanning Calorimetry (DSC) using a Setaram MicroDSC III DSC model. Stainless steel cells were filled by weight with protein samples (8 mg/mL, in the 10 mM Tris-HCl at pH = 8.0, 0.15 M NaCl buffer) and then hermetically sealed and equilibrated for 16 hours at 4 °C. The calorimeter was pre-equilibrated at 5 °C for 10 min, followed by heating from 5 to 50 °C at a scan rate of 0.5 °C/ min. The solvent was the baseline reference. Endothermic peaks with pronounced asymmetry and a gradually decreasing edge were observed for all biopolymers. The transition temperature was determined as the peak temperature (T^{DSC}), while the transition enthalpy and entropies were calculated from the peak area. The transition's enthalpy (ΔH) and entropy (ΔS) were determined by integration of peak area using in-house-developed graphics software. A lysozyme solution was the calibration standard.

Table 2S. Thermodynamic results of the DSC analysis of the aqueous solutions of the UELP4 and HELP biopolymers and their fusion derivatives.

	T^{DSC} °C	Δ*H* kcal·mol^{-1}	Δ*S* cal·K^{-1}mol^{-1}
HELP	36.0	43.2	140
HIn	32.7	7.06	20
UELP4	27.0	9.64	32
U4In	15.8	4.22	13

Statistical analysis of minimal inhibitory (MIC) and minimal biofilm inhibitory (MBIC) concentrations. Nonlinear least squares regression in GraphPad Prism 10.3.1 (464) software (Boston, USA) was used to calculate the peptide concentration (with 95% confidence interval) causing 100% growth inhibition defined MIC and MBIC.

Table 3S. MIC and MBIC of the biopolymers that inhibited 100% bacterial growth. Values are expressed in µM and 95% CI values of MIC and MBIC are shown in brackets.

	***E. coli*, MIC**	***P. aeruginosa*, MIC**	***P. aeruginosa*, MBIC**
HIn	2.8 (2.3-3.4)	5.1 (3.8-7.5)	1.3 (0.7-2.4)
UELP	2.9 (1.8-5.2)	1.3 (0.8-1.8)	0.7 (0.5-1.0)
U4In	0.4 (0.3-0.5)	0.2 (0.1-0.2)	0.3 (0.2-0.4)

Radial Diffusion Assay (RDA). Different amounts of UELP4 were tested, the HELP biopolymer and lysozyme were used as controls. All the reagents and biopolymers were sterilised by 0.22 µm filtration. A single colony from a fresh agar plate was used to inoculate 3 mL of 2.1% (w/v) Mueller-Hinton Broth pH 7.3 (Merck Millipore, Massachusetts, USA). 300 µL of the overnight bacterial culture were diluted in 10 mL of 2.1% (w/v) Mueller-Hinton broth and incubated at 37 °C with continuous shaking (150 rpm) for approximately 2-2.5 hours until an optical density (OD) of approximately 0.5 units was reached. At this point, the bacterial culture was diluted in Mueller-Hinton broth to 0.1 OD units. 450 µL of this bacterial solution was mixed with 25 mL of 10 mM sodium phosphate pH 7.3 containing 0.21% (w/v) Mueller-Hinton powder, 1% (w/v) low EEO-agarose (Sigma-Aldrich, Missouri, USA). The mixture was poured onto 10x10 cm plates (# 82.9923.422, Sarstedt, Numbrecht, Germany) and then cooled to RT to solidify before holes of approximately 2 mm diameter were punched using a glass Pasteur pipette connected to a vacuum pump. 50 µg/µL biopolymer solutions were prepared both for UELP4 and HELP to perform the assay. 2 µL of these solutions were transferred into the holes to deposit 100 µg of biopolymer.
For UELP 4, this solution was further serially diluted 1:1 to load in the holes 50, 25 and 12.5 µg. Lysozyme (2 µg per hole) was used as the positive control. The plates were incubated at room temperature for 1 hour to allow the biopolymers to diffuse, and then they were transferred to 37 °C for 24 hours. Images were captured, and the diameter of the inhibition zones was measured using the ImageJ software [2].

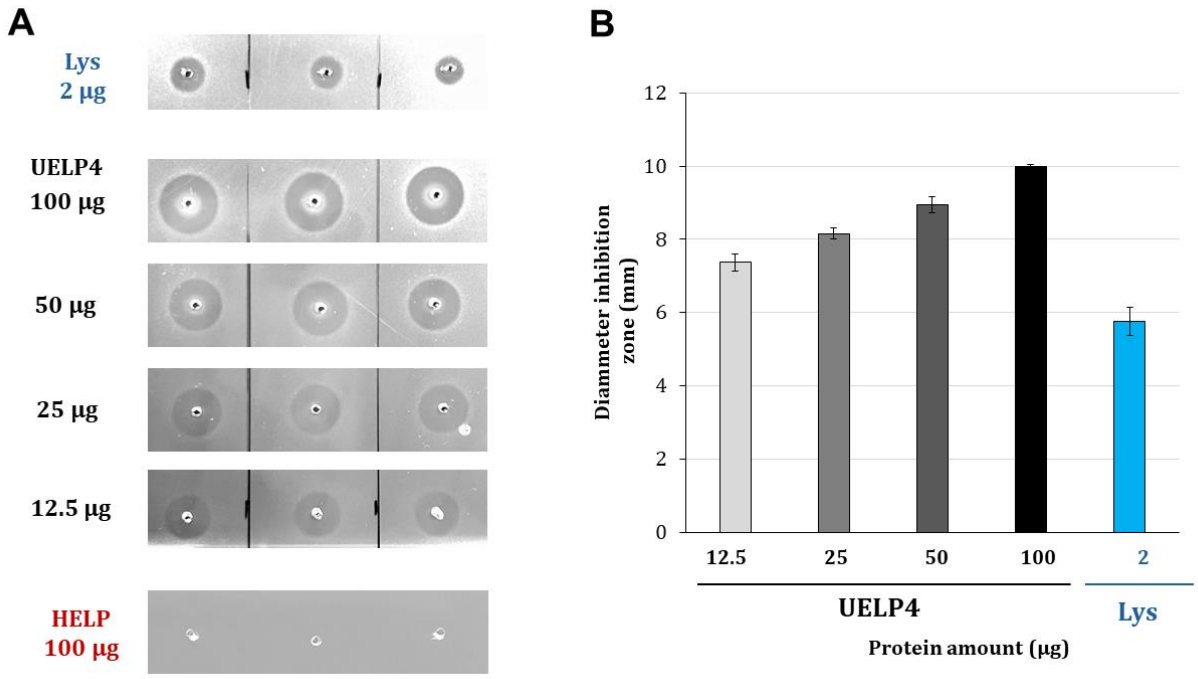

Fig 2S. Antimicrobial activity of UELP4 against the Gram-negative *E. coli* tested by the RDA. (A) Inhibition zones; (B) Diameter of the inhibition zones measured using ImageJ.

References

1- Fraczkiewicz, R., & Braun, W. (1998). Exact and efficient analytical calculation of the accessible surface areas and their gradients for macromolecules. *Journal of computational chemistry,* 19(3), 319-333. https://doi.org/10.1002/(SICI)1096-987X(199802)19:3<319::AID-JCC6>3.0.CO;2-W.

2- Schneider, C.A., Rasband, W.S., Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*. 9(7), 671-675. doi: 10.1038/nmeth.2089.