Supplemental Information

Role of Aromatic Amino Acids in Lipopolysaccharide and Membrane Interactions of Antimicrobial Peptides for use in Plant Disease Control

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Running title: Structural characterization of KYE28 in LPS.

Table S1. Medium- and long-range NOEs (i, ≥ i+5) used to determine the three-dimensional structure of KYE28 in LPS.

KYE28 in LPS	
Medium Range NOE	**Long Range NOE**
Phe23C$^{\alpha}$H-Tyr25HN	Phe19C$^{\beta}$Hs-Tyr25H3
His8C$^{\alpha}$H-Leu10HN	Phe19C$^{\beta}$Hs-Tyr25H2
Thr5C$^{\alpha}$H-Ile7HN	Ile7C$^{\gamma}$H-Tyr25H3
Thr6C$^{\alpha}$H-His8HN	Leu18C$^{\delta}$H-Phe23H2
Tyr2C$^{\alpha}$H-Thr6HN	Thr6C$^{\gamma}$H-Tyr25H2
Ile4C$^{\alpha}$H-Ile7HN	PheC$^{\beta}$Hs-Phe11H2
Ile4C$^{\alpha}$H-His8HN	PheC$^{\beta}$Hs-Phe11H3
Phe23C$^{\alpha}$H-Thr26HN	Leu27C$^{\beta}$Hs-Tyr2H3
Asn22C$^{\alpha}$H-Gly24HN	Leu27C$^{\gamma}$H-Tyr2H3
Thr15C$^{\gamma}$H-Phe19H3	Leu27C$^{\delta}$Hs- Tyr2H3
Leu14C$^{\delta}$Hs-Arg17HN	Leu18C$^{\delta}$H-Phe23H3
Ile7C$^{\gamma}$H-Phe11H2	Thr26C$^{\gamma}$H-Tyr2H3
Thr15C$^{\gamma}$H-Phe19H2	Tyr25C$^{\beta}$Hs-Tyr2H3
Thr15C$^{\beta}$H-Phe19H3	Tyr25C$^{\beta}$Hs-Tyr2H2
Ile7HN-Asn9HN	Leu10C$^{\delta}$Hs-Phe19H2
His16 C$^{\alpha}$H-Phe19HN	Leu10C$^{\delta}$Hs-Phe19H3
Ile7C$^{\gamma}$H-Phe11H2	Phe11H2-Tyr25H2
Tyr25C$^{\alpha}$H-Phe23H2	Leu10C$^{\delta}$Hs-Phe19H2
Phe19C$^{\alpha}$H-Phe23H2	Leu10C$^{\delta}$Hs-Phe19H3
Phe19C$^{\beta}$H-Phe23H2	
Phe11HN-Asn9HN	
Phe19HN-Arg21HN	
Phe23HN-Arg21HN	
Leu27C$^{\delta}$H-Tyr25H2	
Leu27 C$^{\gamma}$H -Tyr25H2	
Phe23H2-Phe19H3	

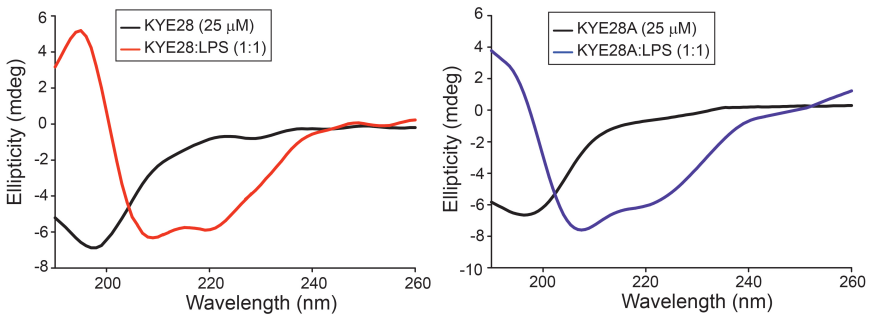


Figure S1. CD spectra of KYE28 (left panel) and KYE28A (right panel) in 10mM phosphate buffer of pH 4.5 (NMR experimental condition) and in the presence of LPS at 25°C. In both cases, the peptides have a random coil conformation in aqueous solution but adopted alpha helical conformation in the presence of LPS.

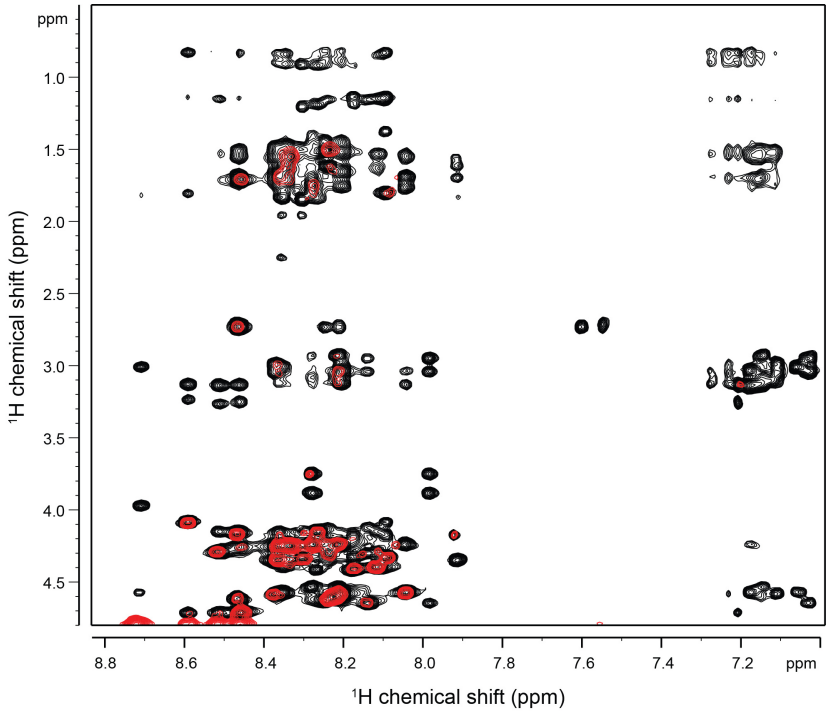


Figure S2. Overlay of the NOESY spectra of KYE28 in aqueous solution (red) and in the presence of LPS micelle (black), showing that the peptide remains unstructured in solution as evident from the appearance of only intra-residual and sequential NOEs. All experiments were performed at 25°C using Bruker Avance III 700 MHz spectrometer, equipped with cryoprobe.

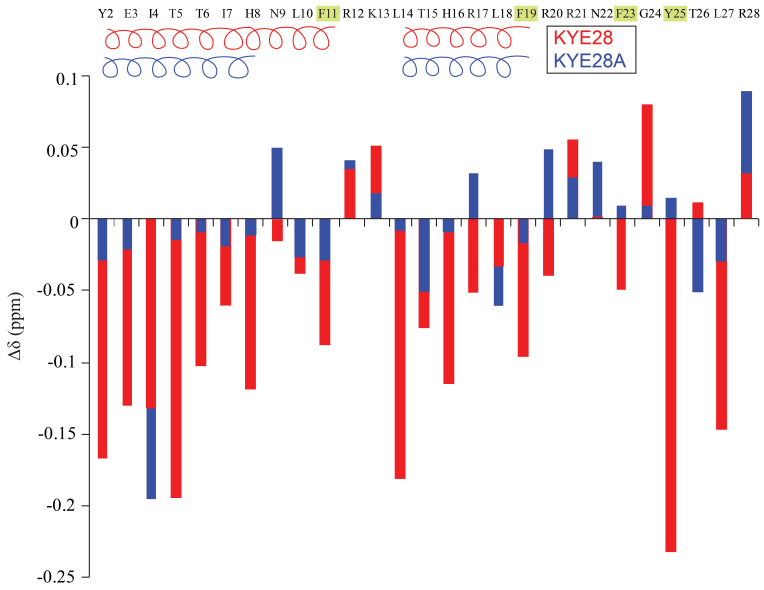
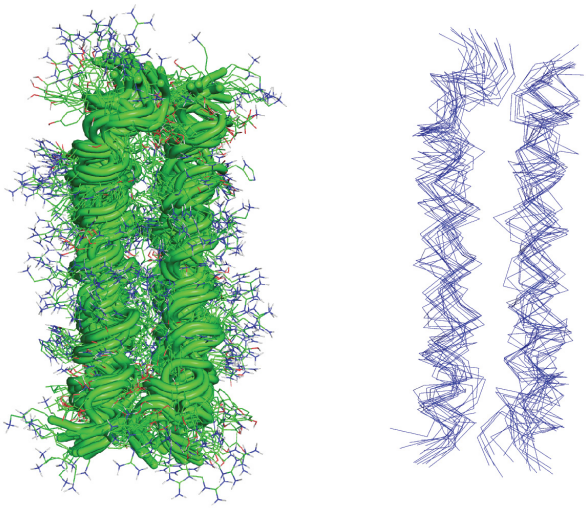
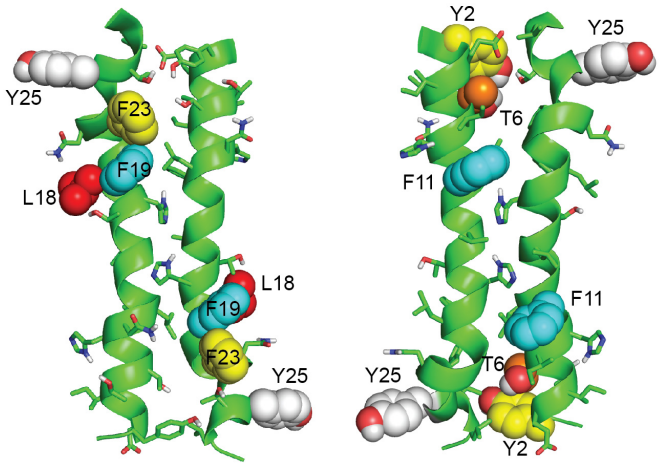


Figure S3. Chemical shift deviations (ΔH^{α}) of the $C^{\alpha}H$ proton from the random coil values for the peptides KYE28 (red) and KYE28A (blue) in LPS. The peptide sequence of KYE28 is shown at the top, with the aromatic residues, mutated to alanine in KYE28A, highlighted in green.

(A)



(B)



(C)

Long range NOE contacts in KYE28 anti-parallel dimer	
NOEs satisfied	**NOEs not satisfied**
Phe19C$^{\beta}$Hs-Phe11H2	Phe19C$^{\beta}$Hs-Tyr25H3
Phe19C$^{\beta}$Hs-Phe11H3	Phe19C$^{\beta}$Hs-Tyr25H2
Leu27C$^{\beta}$Hs-Tyr2H3	Leu18C$^{\delta}$H-Phe23H2
Leu27C$^{\gamma}$H-Tyr2H3	Thr6C$^{\gamma}$H-Tyr25H2
Leu27C$^{\delta}$Hs- Tyr2H3	Leu18C$^{\delta}$H-Phe23H3
Thr26C$^{\gamma}$H-Tyr2H3	Tyr25C$^{\beta}$Hs-Tyr2H3
Leu10C$^{\delta}$Hs-Phe19H2	Tyr25C$^{\beta}$Hs-Tyr2H2
Leu10C$^{\delta}$Hs-Phe19H3	Phe11H2-Tyr25H2
Leu10C$^{\delta}$Hs-Phe19H2	
Leu10C$^{\delta}$Hs-Phe19H3	

Figure S4. Calculated structure of the anti-parallel dimer conformation of KYE28, showing (A) cartoon and line representation of the superimposition of the backbone and side chain atoms of 20 ensemble structures (left panel) and ribbon representation of the backbone superimposition of 20 ensemble structures (right panel). Higher Backbone (N, C^{α} and C') as well as heavy chain RMSD for 20 ensemble structure confirms that the antiparallel dimer is poorly defined. (B) Cartoon and stick representation of single anti-parallel dimer structure of KYE28 where aromatic and hydrophobic residues that share long-range NOE contacts in the trNOESY spectra, but were

not satisfied as either intra-monomeric or intermonomeric NOE contacts in the antiparallel dimer, are highlighted in differently colored spheres. (C) Table showing the important long-range NOEs that were satisfied or not satisfied in the antiparallel dimer conformation marked in blue or red, respectively. As demonstrated, the anti-parallel structure agrees poorly to the experimental results.