Electronic Supplementary Material (ESI) for RSC Advances.

Electronic supplementary information for

Determination of the secondary structure of peptides in the presence of the gram positive bacterium *S. epidermidis* cells†

C. Avitabile[a], L. D. D'Andrea[a], M. Saviano[b], A. Romanelli*[a,c]

[a] Istituto di Biostrutture e Bioimmagini, CNR, via Mezzocannone 16, 80134 Napoli (Italy)

[b] Istituto di Cristallografia, CNR, Via Amendola 122, 70126 Bari (Italy)

[c] Dipartimento di Farmacia, Università di Napoli "Federico II", via Mezzocannone 16, 80134 Napoli (Italy)

Materials and methods

Peptides were obtained following standard protocols[1]. Peptides were purified by RP-HPLC on a Phenomenex Jupiter 10μ Proteo 90 Å (250×10 mm) column and characterized by LC-MS on a Thermo Finnigan instrument equipped with an electrospray source (MSQ) on a Phenomenex Jupiter 5 μ C18 300 Å, (150×4.6 mm) column.

Temporin L (TL): FVQWFSKFLGRIL-NH_2. Calculated mass (Da): 1639.98; found (Da): $[M+2H]^{2+}$ = 820.34; $[M+3H]^{3+}$ = 547.22

TB_KKG6A: KKLLPIVANLLKSLL-NH_2. Calculated mass (Da): 1661.20; found (Da): $[M+1H]^{+}$: 1662.07; $[M+2H]^{2+}$: 831.54; $[M+3H]^{3+}$: 554.70.

Peptides' concentration was determined measuring the absorbance at 205 or 280 nm on a Thermo Fisher Scientific Inc (Wilmington, Delaware USA) Nanodrop 2000c spectrophotometer. The calculated ε for TL and TB_KKG6A are respectively: ε_{280}= 5500 M^{-1} cm^{-1}L for TL and ε_{205}= 39320 M^{-1} cm^{-1} L for TB_KKG6A.

E.coli cells BL21 (DE3) from Novagen and *S. epidermidis* (ATCC 12228) were grown in LB medium at 37°C as described[2, 3]. *S.epidermidis* cells were suspended in Na_2HPO_4 10 mM, KH_2PO_4 2 mM, NaCl 20mM, KCl 2.7 mM, pH 7, centrifuged three times and then resuspended in the same buffer at 0.1 OD_{600}. *E.coli* cells were suspended in 10mM phosphate buffer pH 7, centrifuged three times and then resuspended in the same buffer at 0.1 OD_{600}.

***S.epidermidis* survival assays**.

CFU/mL count

S.epidermidis cells were initially suspended in Na_2HPO_4 10 mM, KH_2PO_4 2 mM, NaCl 20mM, KCl 2.7 mM, pH 7 buffer at 1 OD_{600} . 200μl of the 1 OD_{600} solution were withdrawn and diluted to 2ml in the same buffer solution to a final concentration of 0.1 OD_{600}. Peptides were added to the cell suspension and the mixture (mother) was left at room temperature. Aliquots of the mother were withdrawn, serially diluted in M9 salts buffer (Na_2HPO_4 90 mM, KH_2PO_4 22 mM, NaCl 8,6mM, NH_4Cl 18 mM) and plated onto LB agar plates. The procedure was repeated at different times (t0,t20min, t60min, t80min and t120min) in triplicate. The plates were incubated at 37°C for 24 hours. For the colony count, the ChemiDoc MP system (Bio Rad) and *"the quantity one"* program were employed. The experiment was carried at different peptide concentrations (TL 10 and 15μM, G6AKK 5 and 10μM) and without the peptides (cells in M9 buffer). The CFU/mL value was obtained by the equation:

CFU/mL= number of CFU/mL of diluted solution plated x dilution used.

In the plots we report the cell viability vs time. Cell viability is obtained as follow:

$CFU/mL_{\text{at time 0}}$ x 100/$CFU/mL_{\text{at the desired time}}$.

Results ± SD are reported.

Circular Dichroism

Measurements were carried out on a Jasco J-715 spectrophotometer. Spectra were recorded at 25°C in a 1 cm quartz cell using a 260–198 nm measurement range, 200 nm/min scanning speed, 1 nm bandwidth, 4 s

response time, 1.0 nm data pitch. For the CD experiments, *S.epidermidis cells* were suspended in Na_2HPO_4 10 mM, KH_2PO_4 2 mM, NaCl 20mM, KCl 2.7 mM, pH 7 buffer and *E.coli* cells were suspended in 10mM phosphate buffer pH 7 buffer. Final concentration of both cells was: 0.1 OD_{600}. TL was added to the cells up to a final concentration of 15μM. The concentration of TB_KKG6A was 10μM. CD spectra were recorded every 20 minutes for the first two hours and every two hours up to six hours. Experiments were carried out in duplicate.

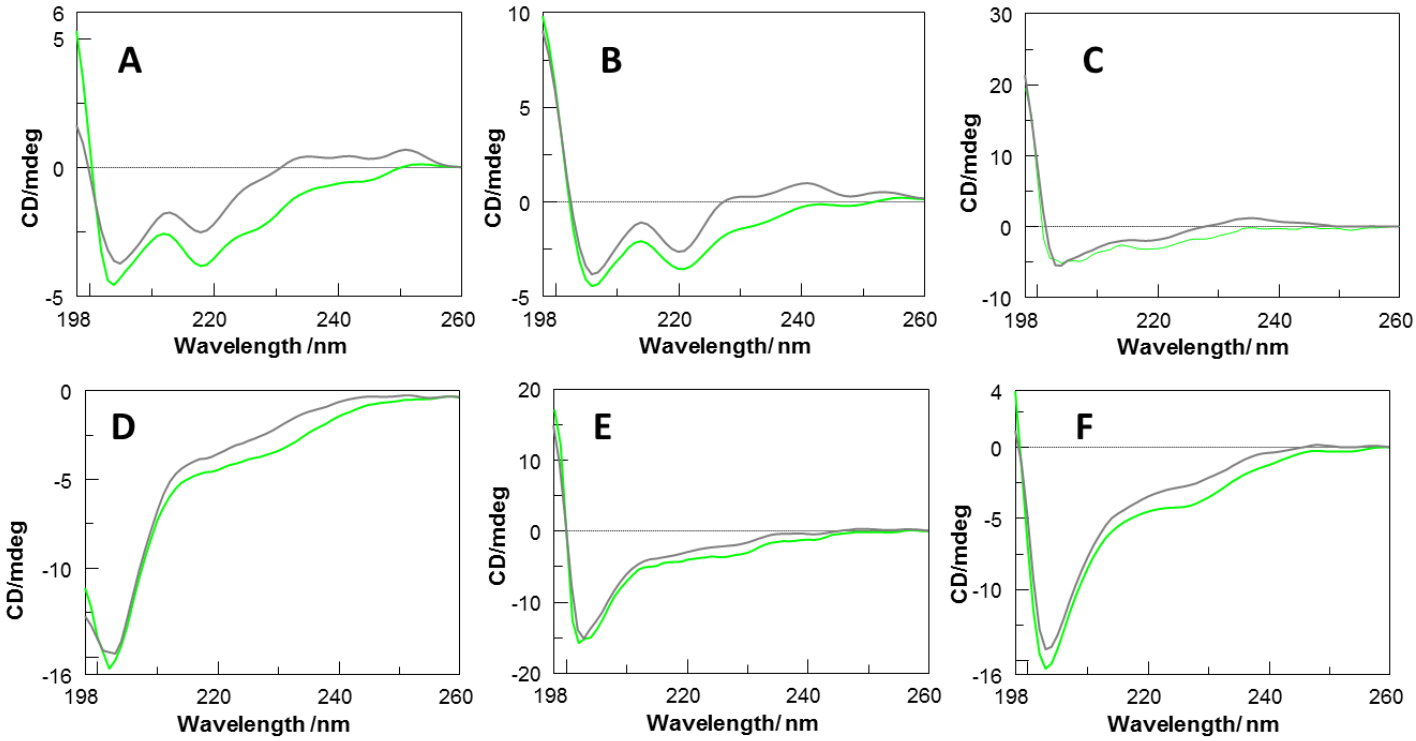

Fig.S1 Comparison of CD spectra recorded for the mixture TL (top panels) or TB_KKG6A (bottom panels) + *S.epidermidis* cells after 20 minutes incubation (A, D), 60 minutes incubation (B, E), 80 minutes incubation (C, F). In green the spectra of the mixture is shown, in grey the spectra obtained upon subtraction of the CD spectrum of cells.

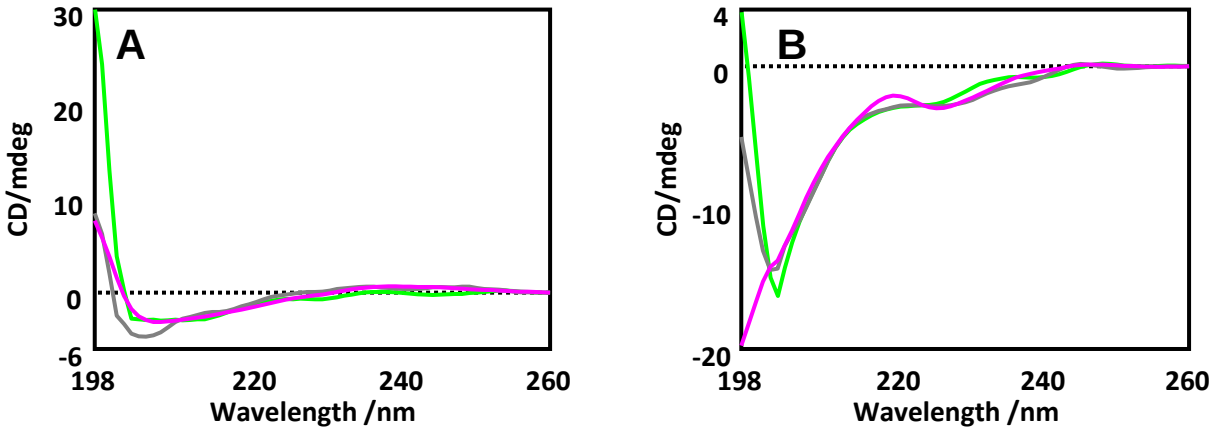

Fig. S2 CD spectra of temporin L 15μM (A) and TB_KKG6A 10μM (B) incubated with *S.epidermidis* cells for 120 minutes (green), 240 minutes (grey) and 360 minutes (magenta). These spectra are representative of one set of experiments and are obtained after subtraction of the CD contribute of *S.epidermidis* cells incubated for the same time as peptides in buffer.

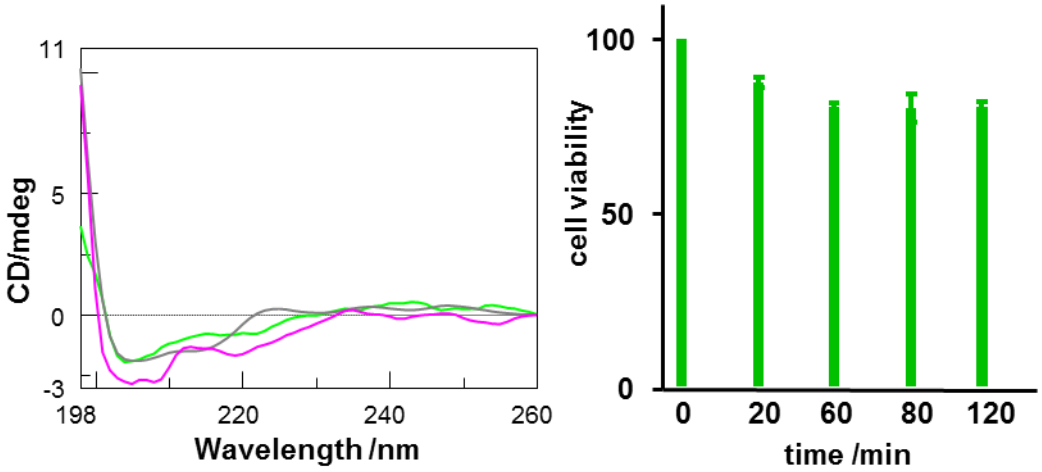

Fig. S3 Left. CD spectra of the peptide temporin L 10μM incubated with *S. epidermidis* cells for 20 minutes (green), 60 minutes (grey) 80 minutes (magenta). These spectra are representative of one set of experiments and are obtained after subtraction of the CD contribute of *S.epidermidis* cells incubated for the same time as peptides in buffer. Right: cell viability expressed as the ratio of CFU/mL at different times x100 and CFU/mL at time zero ±SD.

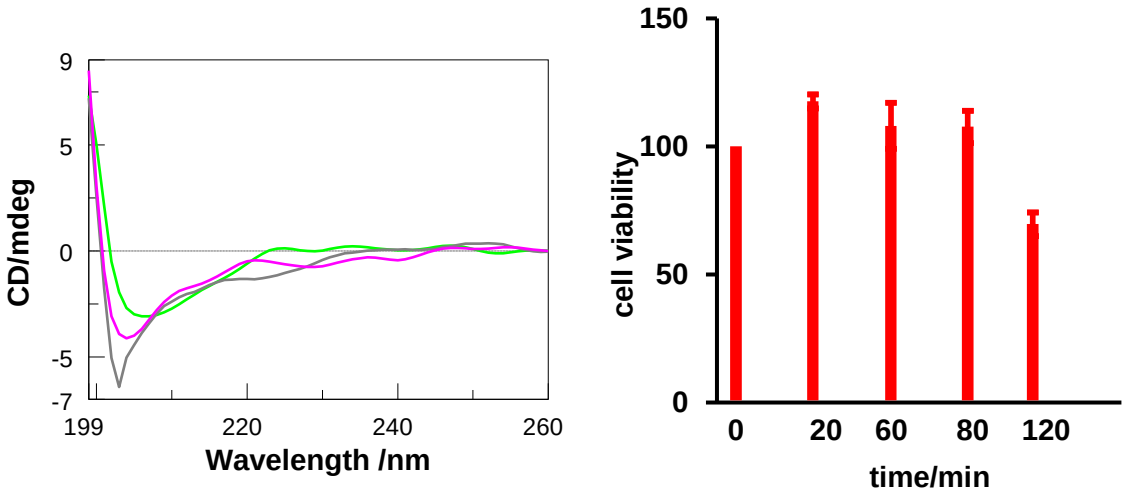

Fig.S4 Left: CD spectra of the peptide TB_KKG6A (5μM) incubated with *S. epidermidis* cells for 20 minutes (green), 60 minutes (grey) 80 minutes (magenta). These spectra are representative of one set of experiments and are obtained after subtraction of the CD contribute of *S.epidermidis* cells incubated for the same time as peptides in buffer. Right: cell viability expressed as the ratio of CFU/mL at different times x100 and CFU/mL at time zero ±SD.

References

1. A. Romanelli, L. Moggio, R. C. Montella, P. Campiglia, M. Iannaccone, F. Capuano, C. Pedone and R. Capparelli, *J Pept Sci*, 2011, **17**, 348-352.
2. C. Avitabile, L. D. D'Andrea and A. Romanelli, *Sci Rep*, 2014, **4**, 4293.
3. G. Malgieri, C. Avitabile, M. Palmieri, L. D. D'Andrea, C. Isernia, A. Romanelli and R. Fattorusso, *ACS Chem Biol*, 2015, **10**, 965-969.