Biophysical Journal, Volume *117*

Supplemental Information

Inactivation of Bacteria by γ-Irradiation to Investigate the Interaction with Antimicrobial Peptides

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Infrared spectroscopy Method

The overnight bacterial cell culture of *S. enterica* R60 was diluted 1:50 in a fresh LB medium. After attaining the middle exponential phase (3.5 h), the number of viable bacteria was quantified. OD_{600} was measured and compared to a standard curve to relate the OD values to CFU/ml. The bacterial cell culture was centrifugated at 4610 rpm, hold for 20 min at 4 °C, then the pellet was resuspended with 20 mM HEPES pH 7.4 buffer, supplemented with 150 mM NaCl to get a final concentration of 1.0×10^9 CFU/ml. An amount of 1 ml of the resulting solution was either non-treated, γ-irradiated (1000 Gy) or heat-killed (submerged in 100 mL of boiling water for 10 min). After the treatment, bacterial suspension was centrifugated at 13200 rpm during 5 min and resuspended in approximately 20 µL of buffer. 10 µL of the bacterial pellet was spread on a CaF_2 crystal (Ø 19-0.2 mm × 3 ± 0.1 mm) and the buffer was slowly removed with $N_{2(g)}$, without total drying. The infrared spectroscopic measurements were performed on an IFS-55 spectrometer (Bruker, Karlsruhe, Germany). Consecutive heating-scans were performed automatically from 5°C to 70°C with a heating rate of 0.6°C min^{-1}. Every 3°C, 200 interferograms were accumulated, apodized, Fourier-transformed, and converted to absorbance spectra. The resolution, aperture and velocity were 2 cm^{-1}, 10 mm and 10 kHz respectively. The symmetric stretching vibration of the methylene band ν_s (-CH_2-) localized in the range 2850 to 2853 cm^{-1} was plotted versus temperature. Phase transition temperatures were derived by determination of the maximum of the first derivative of the heating scans by using OriginPro8 software mathematical tools.

Table S1. Thermodynamic parameters of binding of PMB and LL-32 to LPS Ra aggregates. The values represent the mean of three independent experiments with the standard deviation (s.d).

Thermodynamic parameters	**Polymyxin B**	**LL-32**
n (mol ratio)	0.74 ± *0.08*	0.66 ± *0.05*
K_D (nM)	458.5 ± *144*	107.3 ± *71.4*
ΔH (kJ/mol)	- 25.1 ± *4.2*	- 41.5 ± *0.5*
$-T\Delta S$ (kJ/mol) [a]	-12.7 ± *5.1*	- 0.2 ± *1.6*
ΔG (kJ/mol) [b]	- 37.8 ± *0.9*	- 41.8 ± *1.7*

[a] Entropy changes of binding were calculated with $\Delta G = \Delta H - T\Delta S$

[b] Free energy changes were calculated according to $\Delta G = -RTlnK_A$

The peptides underwent a conformational change upon the binding which increased the molecular interaction between the residues, facilitating the initial electrostatic interaction with LPS Ra. As a result, the gain in translational and rotational freedom of the water molecules generated an increase in entropy that helped the peptide to intercalate into the hydrophobic core of the aggregates.

Table S2. Antibacterial activity of peptides against Gram-negative rough mutant *S. enterica* R60*.* The minimal inhibitory concentration (MIC) values are given in µg/mL. The assay was performed in 20 mM HEPES pH 7.4 or with 150 mM NaCl (parenthesis), each supplemented with 10 % of LB.

Bacterial strain	**Polymyxin B**	**LL-32**
S. enterica R60	< 2 (2)	32 (32)

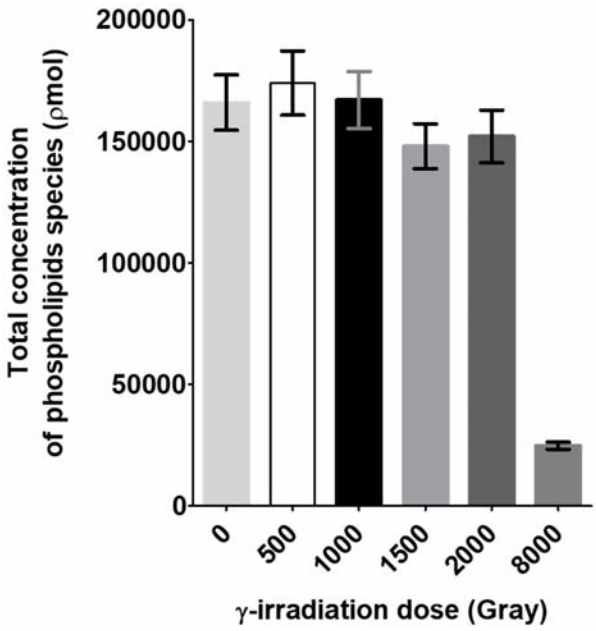

Fig. S1. *Influence of γ-irradiation on the total phospholipid species in S. enterica R60.* Lipidomics measurements of the total phospholipids of identified PE and PG major species in *S. enterica* R60 at different γ-irradiation doses. The quantitative determination was performed using 1,2-di-O-phytanyl-sn-glycero-3-phosphoethanolamine as internal standard and MS^1- intensities for normalization. The total phospholipid concentrations are shown in ρmol. Mean +/- s.d of two independent experiments with two technical replicates are shown.

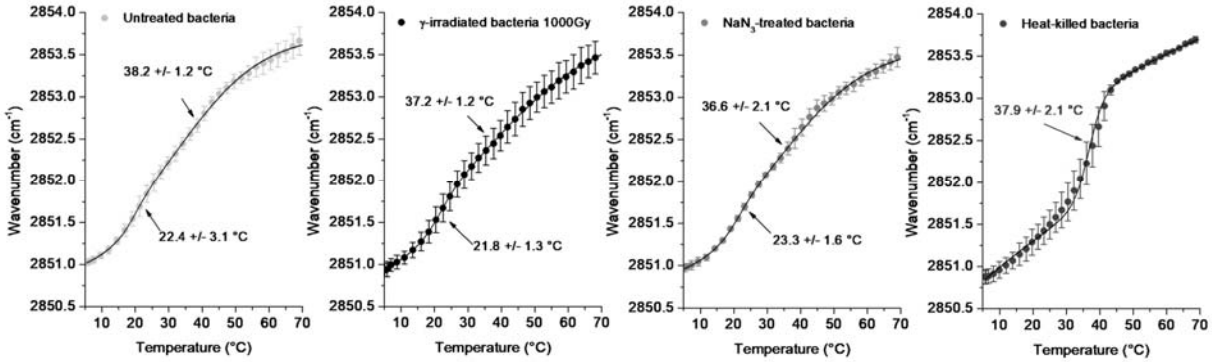

Fig. S2. Gel to liquid-crystalline phase transitions in *S. enterica* R60 untreated and γ-irradiated bacteria. The results demonstrate that the gel to liquid-crystalline phase transitions of the hydrocarbon chains were similar for (**A**) untreated bacteria, (**B**) γ-irradiated bacteria and NaN_3-treated bacteria (**C**). (**D**) When the bacteria were heat-killed, the phase transition at the lower temperature was not observed. The presented data show the peak position of the symmetric stretching vibrational band of the methylene groups ν_s (CH_2) versus temperature. The mean +/- s.d of three independent experiments are given in the errors bars.

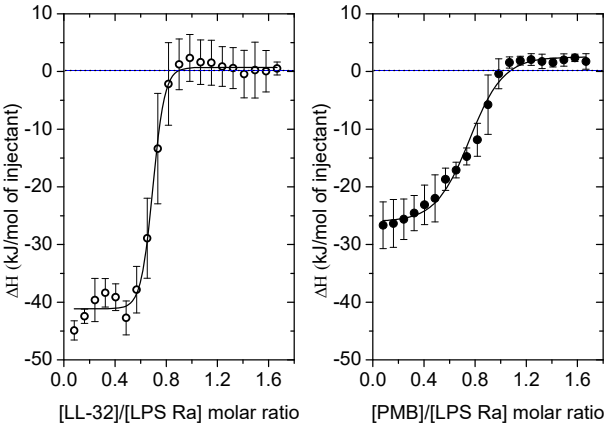

Fig. S3. Binding curves for the interaction of PMB and LL-32 with LPS Ra chemotype aggregates using isothermal titration calorimetry. (**A**) Binding curve of LL-37 with LPS Ra. (**B**) Binding curve of PMB with LPS Ra. The heat of the reaction (ΔH) is presented as a function of the number of peptide / LPS Ra molar ratios. The thermodynamic parameters calculated form these experiments are presented in Table S1. The mean +/- s.d of three independent experiments are shown in the errors bars.

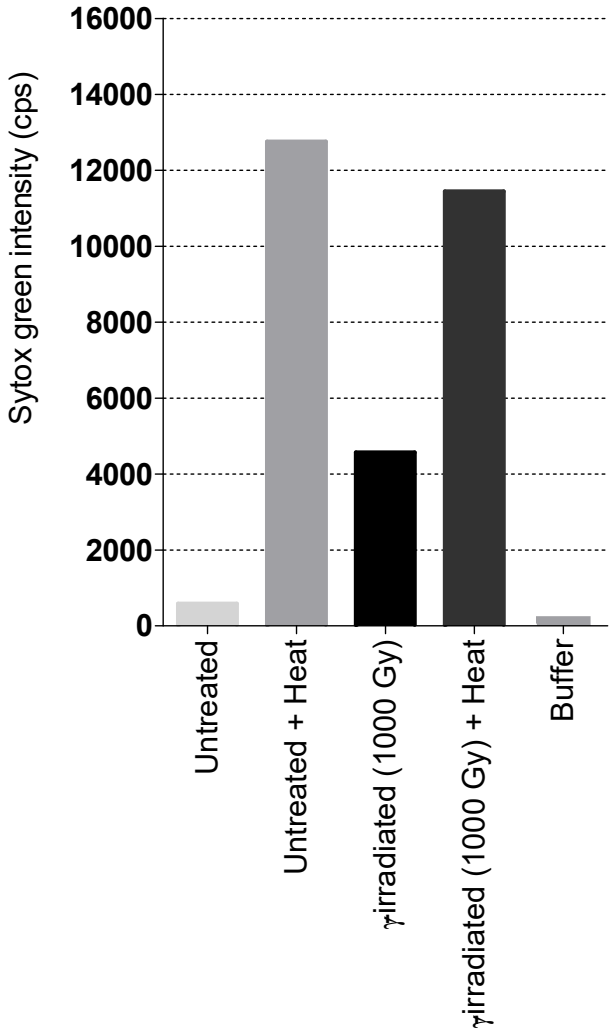

Fig S4. Sytox green uptake by *S. enterica* R60 nucleic acids under different experimental conditions. *S. enterica* R60 untreated bacteria were treated with heat (95˚C, 10 min and 2 min sonification), γ-irradiated (1000 Gy) and γ-irradiated (1000 Gy) plus heat (95˚C, 10 min and 2 min sonification). 10 µl of a stock SYTOX solution (50 µM of SYTOX green stain) was added to 1.0×10^7 CFU/mL. The fraction of permeabilized cells in the population was quantified by fluorometry. Sytox green stain was excited at 480 nm, and the intensity of the emission light was detected at 530 nm. Fluorescence emission was measured after 60 min. Two independent experiments were performed.