Supplementary Material

Supplementary Figure S1. Membrane potential of *S. aureus* SG511-Berlin in half-concentrated MHB in absence (A) and presence (B) of 10 mM glucose. RTDs were added in concentrations corresponding to 10× MIC. The pore-forming peptide nisin was used as a control. Arrows indicate the moment of peptide addition.

Supplementary Figure S2. Localization of Pep5-Cy3 in *S. aureus* SA113. Cells were grown to exponential phase, incubated with Pep5-Cy3 for 5 min and inspected by fluorescence microscopy.
**Supplementary Figure S3.** Carboxyfluorescein (CF) release from liposomes made of DOPC and 0.5 mol% LTA. RTDs (A) and Pep5 (B) were added at 1 µM. Marker release was expressed relative to the amount of CF released after addition of Triton X-100 (100% efflux). Pure DOPC vesicles were used as a control.

**Supplementary Figure S4.** Transmission electron microscopy of *E. coli* BW25113 treated with 10× MIC RTD-2. (A) Untreated control cells; (B) Cells treated for 15 min; (C) Cells treated for 30 min. Scale bar: 0.5 µm.
Supplementary Experimental Methods

**Supplementary Table S1.** Strains used in this study.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant characteristic(s)</th>
<th>Reference/Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> SG511-Berlin</td>
<td>Mutation in gene <em>graS</em></td>
<td>[1]</td>
</tr>
<tr>
<td><em>S. aureus</em> SA113</td>
<td>Derivative of <em>S. aureus</em> NCTC 8325</td>
<td>[2]</td>
</tr>
<tr>
<td><em>S. aureus</em> SA113 ∆atl</td>
<td>∆atlA deletion mutant of strain SA113 (∆atlA::spc)</td>
<td>[3]</td>
</tr>
<tr>
<td><em>S. simulans</em> 22</td>
<td>Indicator strain</td>
<td></td>
</tr>
<tr>
<td><em>S. carnosus</em> TM300</td>
<td>Indicator strain</td>
<td>[5]</td>
</tr>
<tr>
<td><em>E. coli</em> BW25113</td>
<td>K-12 strain</td>
<td>[6]</td>
</tr>
</tbody>
</table>

**CF Efflux**

Vesicles were made of pure DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine; Avanti polar lipids, Alabaster, AL, USA) or DOPC supplemented with 0.5 mol% LTA (Sigma-Aldrich, Taufkirchen, Germany) referring to the total amount of phospholipids. For this, 4 μM DOPC were mixed with LTA and the solvent was evaporated under a nitrogen stream. Then, the lipids were resuspended in 600 μL buffer (50 mM MES-KOH; 100 mM K₂SO₄; pH 6 or 10 mM Tris-HCl; 0.85% NaCl; pH 7.2) containing 50 mM carboxyfluorescein (CF). Unilamellar vesicles were prepared by the extrusion technique (polycarbonate filter, pore size 0.4 μm; Whatman™, Dassel, Germany). Liposomes were separated from unencapsulated CF by gel filtration using sephadex G-50 (Sigma-Aldrich).

The CF-loaded vesicles were diluted in 1.5 mL buffer (see above) at a final concentration of 25 μM phospholipid on a phosphorous base. The fluorescence intensity was measured at 520 nm (excitation at 492 nm) on a RF-5301 spectrophotometer (Shimadzu, Duisburg, Germany) for 3 min at RT. Peptides were added after 25 s at concentrations of 1 μM. To determine 100% marker release, 20 μL of 20% Triton X-100 (v/v) were added at the end of each measurement.

**Fluorescence Microscopy**

Pep5 was fluorescently labeled using the Cy3 Mono Reactive Dye Pack (GE Healthcare, Buckinghamshire, UK) according to the manufacturer’s instructions.

For localization of Pep5, *S. aureus* SA113 was grown to mid-exponential phase, incubated with Pep5-Cy3 and DAPI (4',6-Diamidino-2-phenylindol; 0.25 μg/mL) for 5 min at 37 °C and subsequently washed three times with SPB (10 mM, pH 7.5). Then, 10 μL of cell suspension were applied on glass slides covered with 1% agarose and examined with a DMRB fluorescence microscope (Leica, Wetzlar, Germany).
References


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