

SUPPLEMENTARY INFORMATION

Inhibition of *Staphylococcus aureus* cell wall biosynthesis by desleucyl-oritavancin: a quantitative peptidoglycan composition analysis by mass spectrometry

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Running title: LC-MS analysis of oritavancin-treated *S. aureus*

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Muropeptides	Cross-linking efficiency (ρ_{CL}) [†]		P value ^{**}
	des-Ori (0 $\mu\text{g/mL}$) [*]	des-Ori (10 $\mu\text{g/mL}$) [*]	
All muropeptides	0.636 \pm 0.005	0.630 \pm 0.002	0.024
With a terminal pentaglycine bridge structure	0.632 \pm 0.006	0.6274 \pm 0.002	0.092
Without a pentaglycine terminal bridge	0.686 \pm 0.003	0.667 \pm 0.010	0.004
Tripeptide stem structure	0.649 \pm 0.016	0.625 \pm 0.023	0.053
Tetrapeptide stem structure	0.593 \pm 0.016	0.625 \pm 0.002	0.003
Pentapeptide stem structure	0.639 \pm 0.003	0.631 \pm 0.004	0.008

[†] Cross-linking efficiencies calculated based on equation in Fig. 3b.

^{*} All error bars represent 95% confidence interval (n = 3).

^{**} Unpaired Student's t-test.

Table S1. Cross-linking efficiency of muramidase digested PG from isolated cell walls of *S. aureus* grown in absence or in presence of des-Ori (10 $\mu\text{g/ml}$) classified based on PG-stem modifications.

Muropeptide Acetylation states	Cross-linking efficiency (ρ_{CL}) [†]		P value ^{**}
	des-Ori (0 $\mu\text{g/mL}$) [*]	des-Ori (10 $\mu\text{g/mL}$) [*]	
-1	0.631 \pm 0.015	0.607 \pm 0.023	0.056
0	0.628 \pm 0.008	0.622 \pm 0.005	0.117
1 (or higher)	0.642 \pm 0.003	0.638 \pm 0.001	0.011

[†] Normalized percentile composition based on the sum of integrated XIC of selected ions.

^{*} All error bars represent 95% confidence interval (n = 3).

^{**} Unpaired Student's t-test.

Table S2. Cross-linking efficiency of muramidase digested PG from isolated cell walls of *S. aureus* grown in absence or in presence of des-Ori (10 $\mu\text{g/ml}$) classified based on acetylation state.

Muropeptides		Composition (%) [†]		P value ^{**}
PG-stem length	Acetylation state	des-Ori (0 µg/mL) [*]	des-Ori (10 µg/mL) [*]	
3	-1	9.764 ± 2.964	15.863 ± 2.964	0.010
	0	19.450 ± 10.808	10.733 ± 0.808	0.063
	1	70.787 ± 10.121	73.405 ± 2.443	0.469
4	-1	2.838 ± 0.356	5.227 ± 3.638	0.106
	0	35.386 ± 6.176	42.133 ± 3.375	0.038
	1	61.776 ± 5.895	52.641 ± 0.295	0.008
5	-1	6.063 ± 1.487	5.674 ± 2.598	0.700
	0	39.892 ± 2.287	40.235 ± 3.910	0.821
	1	54.045 ± 2.869	54.091 ± 1.984	0.968

[†] Normalized percentile composition based on the sum of integrated XIC of selected ions.

^{*} All error bars represent 95% confidence interval (n = 3).

^{**} Unpaired Student's t-test.

Table S3. Normalized PG composition of muramidase digested cell walls isolated from *S. aureus* grown in absence or in presence of des-Ori (10 µg/ml) classified based on PG-stem length and acetylation state.