Supporting Information

Nutrient-dependent Structural Changes in *S. aureus* Peptidoglycan Revealed by Solid-State NMR Spectroscopy

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Table S1. Additional cell-wall properties at different growth stages

OD	peptidoglycan yield / cell[a]	Incorporation of L-[ε-^{15}N] Lys in peptidoglycan[b]
0.7 (3.5 h)	16%	1.00
2.0 (5.2 h)	18%	1.25
3.6 (8 h)	20%	1.48
4.0 (12 h)	21%	1.43

[a] Percentages of peptidoglycan (dry mass) isolated from whole cells (dry mass). [b] Values are from the sums and calibrated integrals of ^{15}N CPMAS spectra of L-[ε-^{15}N]Lys-labeled peptidoglycan normalized by the dry weight of whole cells used for the isolation.

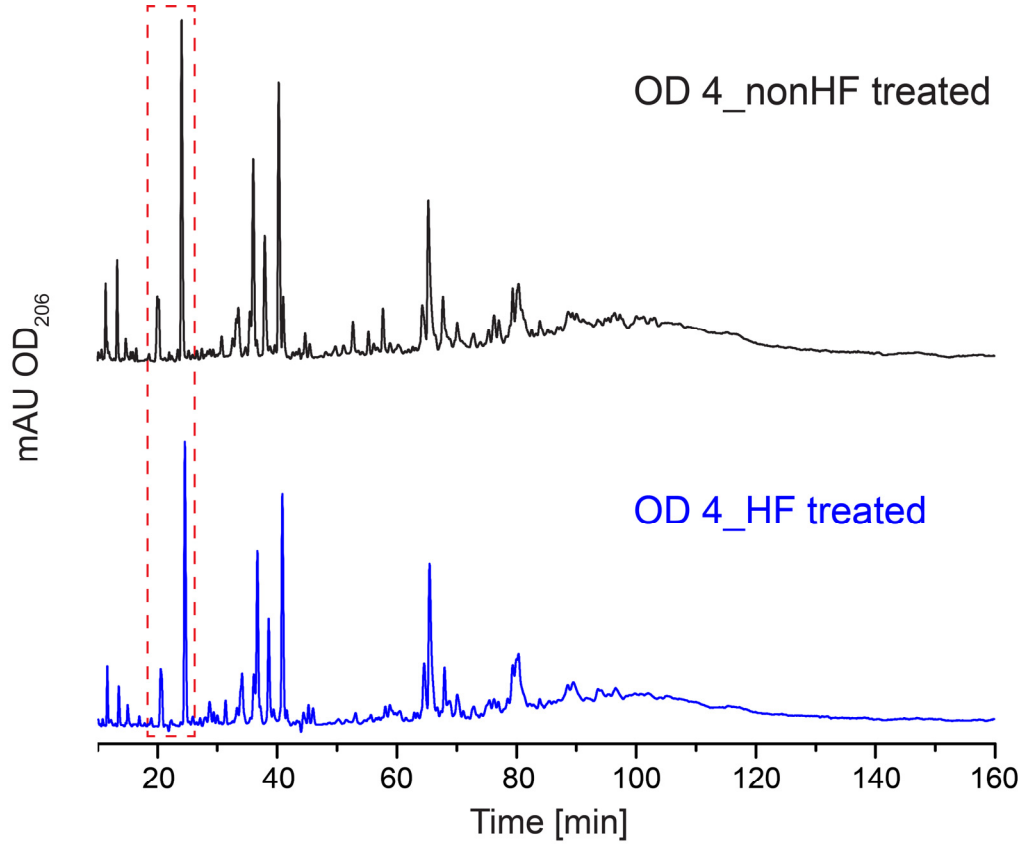

Figure S1. Muropeptide profiles of OD 4 peptidoglycan treated with and without hydrofluoric acid. Isolated peptidoglycan was treated with mutanolysin and resolved with reversed-phase HPLC as described in "Materials and Methods". The major features are independent of the hydrofluoric acid treatment, particularly the peaks highlighted in the red dashed box that are characteristic of the OD 4 peptidoglycan (Figure 6a). In general, the hydrofluoric acid treatment gives a cleaner profile with certain peaks removes, which were identified as O-acetylated peptidoglycan fragments by LC-MS (Table S2).

Table S2. Additional monomeric muropeptides detected in non-HF treated OD 4 peptidoglycan

m/z	Proposed structure[a]
1011.5 (969.5+42)	GlcNAc-(1,6-N,O-diacetyl)MurN-AQKAA
1082.5 (1040.5+42)	GlcNAc-(1,6-N,O-diacetyl)MurN-AQK(A)AA
869.5 (827.4+42)	GlcNAc-(1,6-N,O-diacetyl)MurN-AQK

[a] Based on the increase of 42 m/z units (due to acetylation) and MS/MS data which shows a loss of 203 units (GlcNAc) instead of 245 units (acetylation on GlcNAc) during fragmentation for each species listed in this table, we attributed the gain of 42 units to the O-acetylation of MurNAc. This is consistent with a previous known modification of peptidoglycan at C6 of MurNAc in *S. aureus* (*1*).

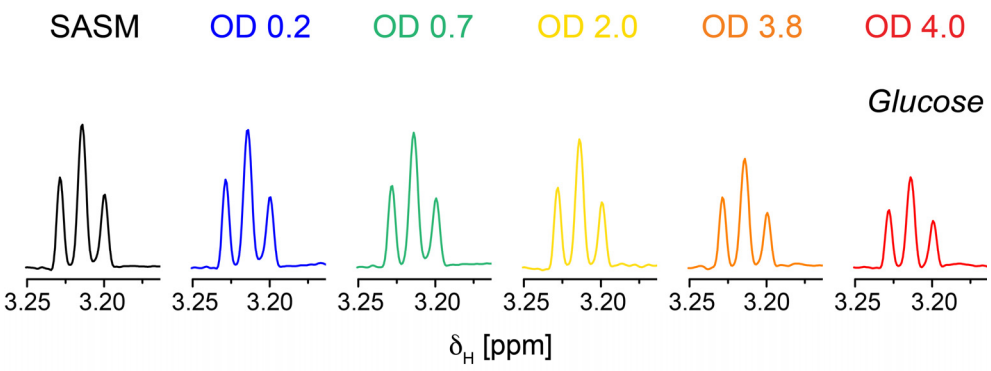

Figure S2. Glucose level in the media (SASM). Glucose level was monitored by solution NMR using natural abundance glucose. Cultures from designated growth stages (OD_{660}) were sterile filtered to remove the cells, and ^{1}H-^{13}C HSQC spectra were acquired (without an array in the F1 dimension). The glucose region of the ^{13}C-selected ^{1}H NMR spectra obtained at different growth stages is shown. More than 50% of the glucose is still present in the stationary phase culture.

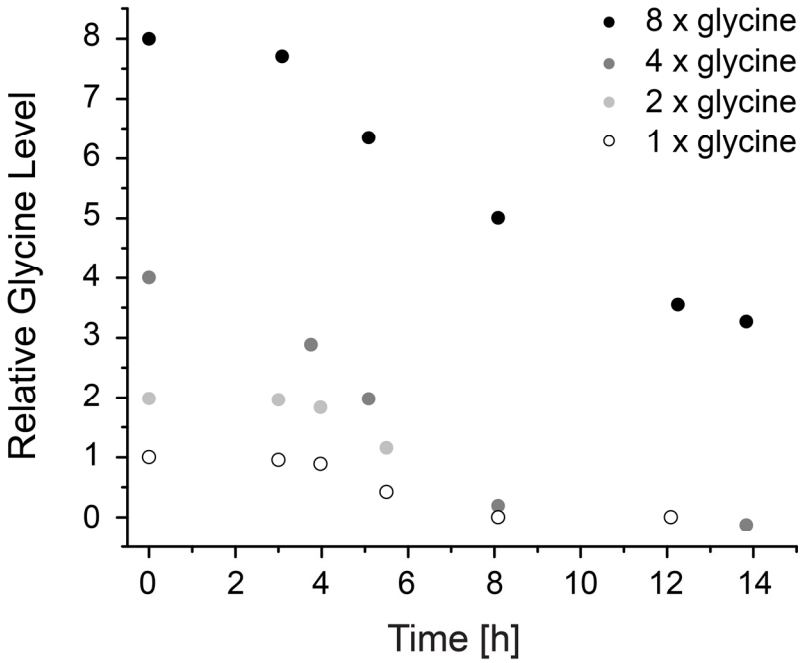

Figure S3. Glycine level in the media supplemented with various concentration of glycine. Glycine levels were monitored by solution NMR using [2-^{13}C]glycine. Glycine is depleted at stationary phase for *S. aureus* cultures grown in SASM with up to 4x glycine, while there is still excess glycine available at stationary phase in SASM with 8x glycine.

Reference:

1. Vollmer, W. (2008) Structural variation in the glycan strands of bacterial peptidoglycan, *FEMS Microbiol. Rev. 32*, 287-306.