Supporting Information for

Peptidoglycan and Teichoic Acid Levels and Alterations in *S. aureus* by Cell-Wall and Whole-Cell NMR

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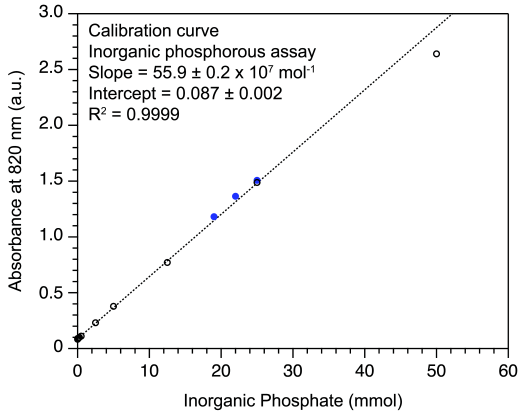
This pdf file includes:

Supplementary Figures (Figures S1 to S5)



	nmol Pi / mg material	Weight % WTA
Cell Wall n = 4	459 ± 77	19 ± 3
Teichoic acid n = 5	2070 ± 295	90 ± 10

Figure S1. Inorganic phosphate determination in MW2 cell. To determine the amount of teichoic acid present in cell walls using quantitative chemical analysis, cell walls were chemically oxidized, and organic materials were then burned away. The residual inorganic phosphates were hydrolyzed and complexed with molybdate to enable colorimetric detection of phosphate content. Left: A standard curve was generated from a series of phosphoric acid standards (black). Cell wall analyte is in blue. Right: Calculations of phosphorous content suggest 19 wt% of the cell wall is teichoic acid, consistent with NMR measurements.

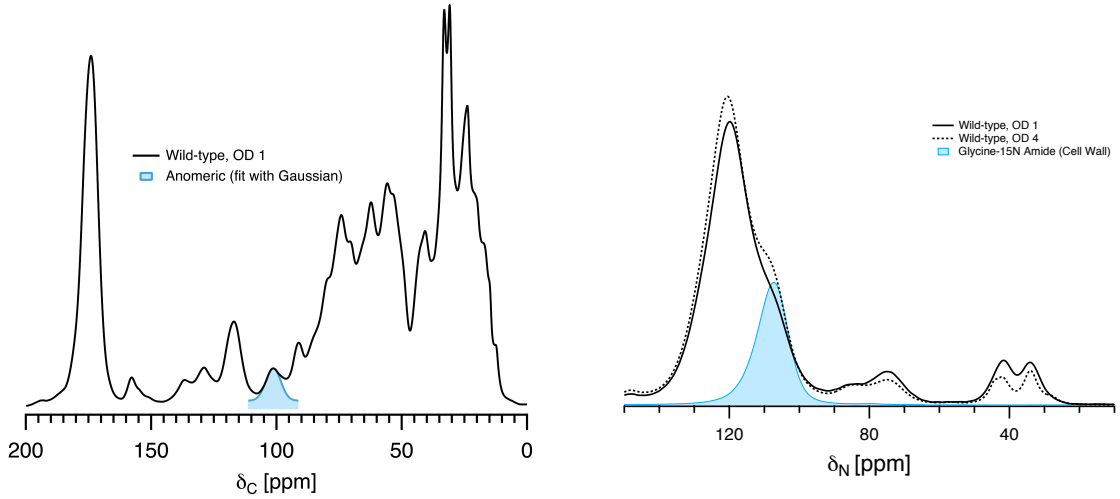


Figure S2. Peak area determination from whole cell spectra. Left: A Gaussian curve (blue) was fit to the anomeric carbon at 101 ppm of the ^{13}C CPMAS whole cell spectrum of wild-type cells harvested at OD 1.0. Right: The glycyl amide contribution to whole cells is clear from a cell wall spectrum isolated from cells selectively labeled with [^{15}N] glycine (blue). This experimental spectrum was used to fit the amide shoulder at 109 ppm of a ^{15}N CPMAS spectrum of wild-type, OD 1.0 whole cells (black). In both cases, the fits were integrated to measure the relative contributions of anomeric carbons and cell wall glycyl amides to whole cell ^{13}C and ^{15}N spectra, respectively. These spectra are representative of the procedure used to quantify all spectral regions tabulated in Figure 4C.

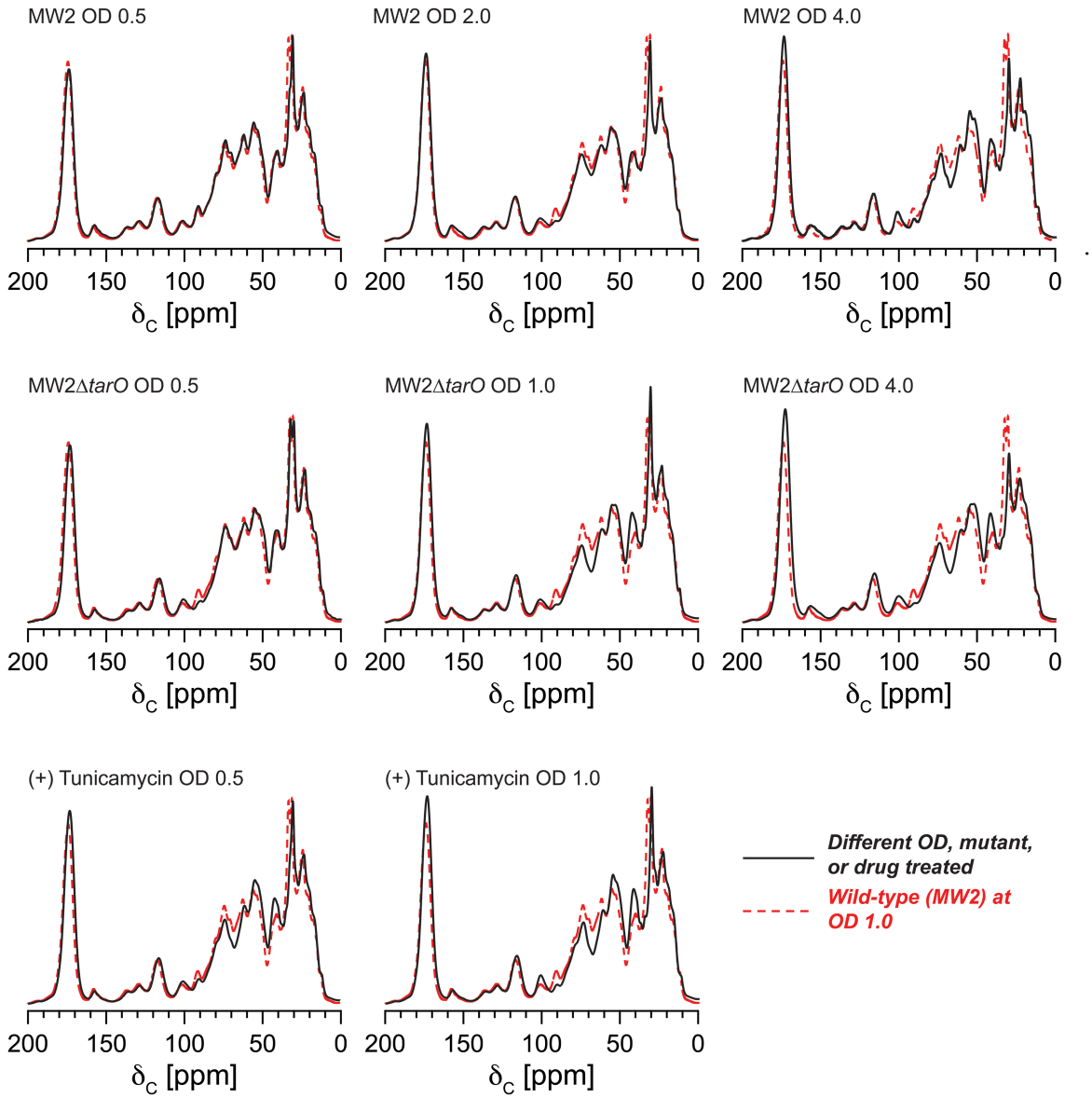


Figure S3. Changes in whole-cell ^{13}C NMR spectra as a function of cell density and tunicamycin treatment. (A) In wild-type *S. aureus* MW2, ribitol phosphate peaks decrease in intensity as cell density increases from OD 0.5 to 4.0, indicating that WTA becomes less abundant by overall carbon composition. The anomeric carbon peak, on the other hand, increases in intensity, indicating increased PG production from OD 0.5 to 4.0. (B) The same trend is observed for *S. aureus*Δ*tarO*. Without WTA, only lipoteichoic acid contributes to the ribitol phosphate peaks, thus lipoteichoic acid also decreases with respect to peptidoglycan as cells enter stationary phase. (C) Cells treated with tunicamycin and harvested at OD 0.5 began to reveal the changes observed when harvested at OD 1.0, as in Figure 5 and reproduced above. Reduced teichoic acid levels are indicated by a reduction in ribitol phosphate peaks as compared to untreated cells. The reduction in PG observed for cells harvested at OD 1.0 was also evident.

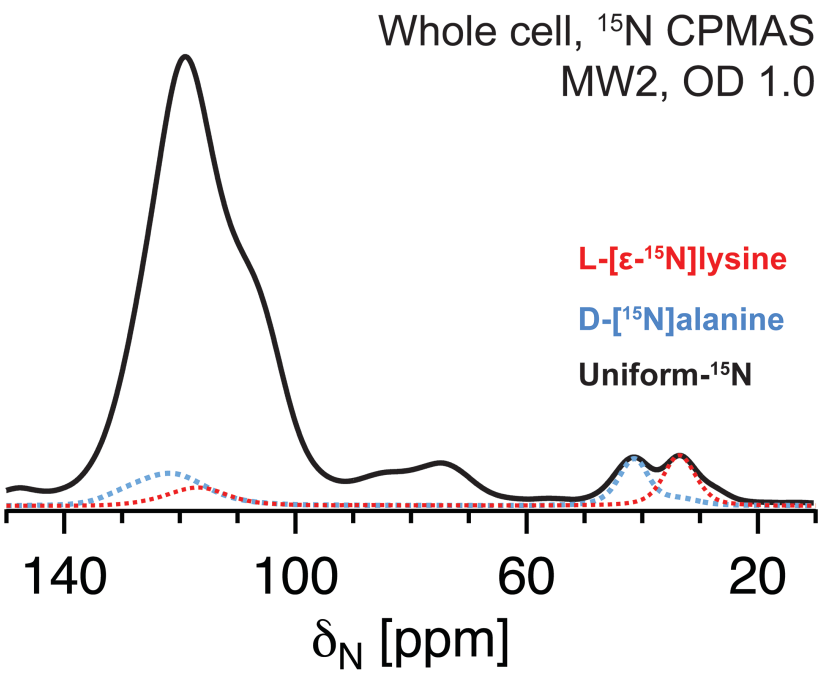


Figure S4. Selective amino acid labeling of S. aureus cells. ^{15}N CPMAS of whole cells grown with either D-[^{15}N]alanine (blue) or L-[ε-^{15}N]lysine (red). The specific isotopic labeling preparations support the resonance assignment of the WTA D-alanyl amine at 42 ppm in uniformly labeled whole cells (black). The lysyl amines appear at 34 ppm. Each spectrum was acquired with 32,768 scans**.**

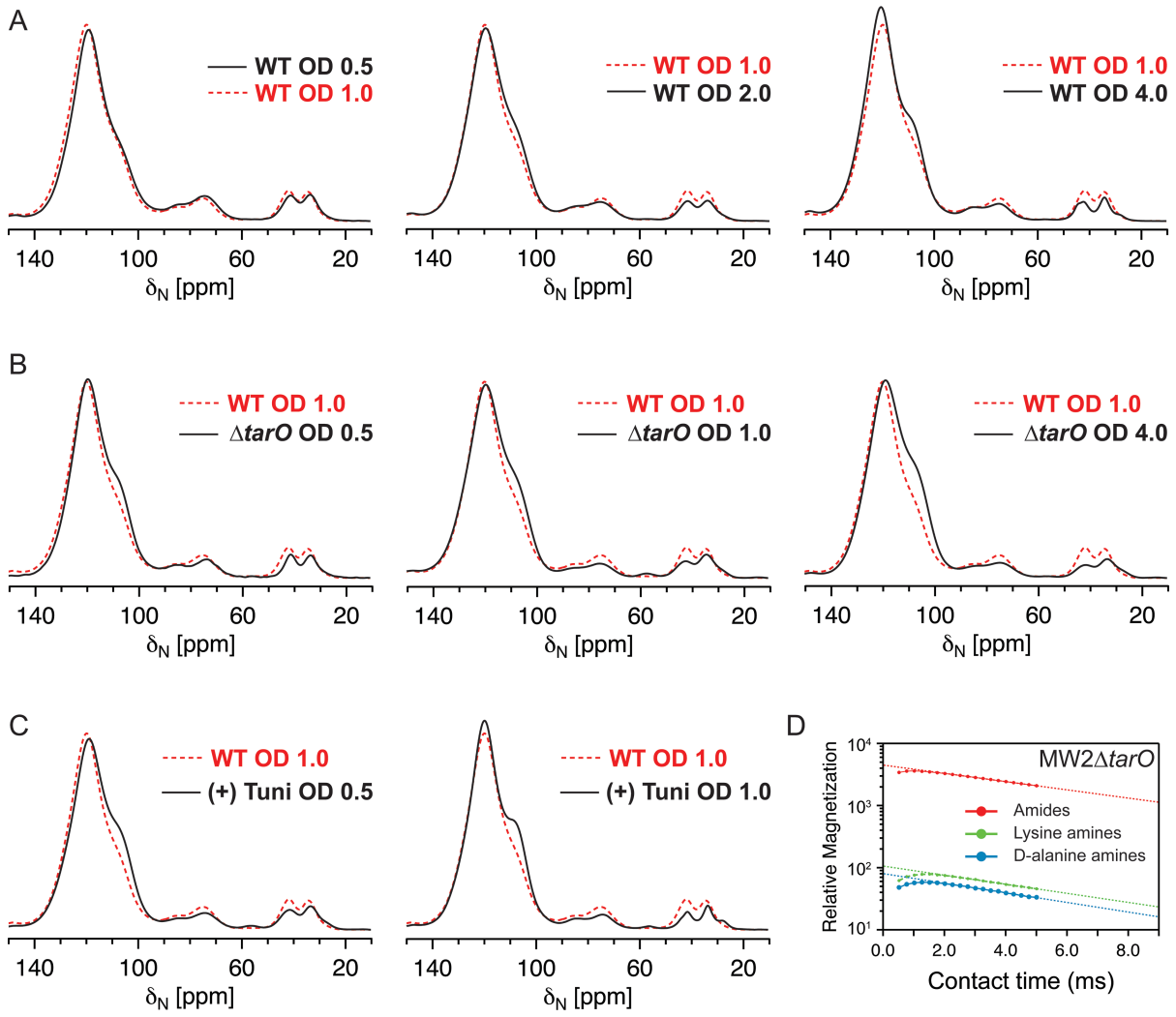


Figure S5. Changes in whole-cell ^{15}N NMR spectra as a function of cell density and tunicamycin treatment. (A) In wild-type *S. aureus* MW2, the intensity of the D-alanyl amine peak at 42 ppm decreases as cells enter stationary phase, indicative of reduced teichoic acid levels. The amide shoulder intensity, centered at 105 ppm, increases due to increased PG production. (B) ^{15}N CPMAS spectra for MW2Δ*tarO* exhibit a similar trend. For increasing OD, PG levels increase and teichoic acid levels – in this case, lipoteichoic acids – fall, as measured by the glycyl amide and D-alanyl amine intensities, respectively. (C) Cells treated with low concentrations of tunicamycin, likewise show reduced D-Ala amine levels compared to untreated cells, and a corresponding increase in the relative abundance of glycyl amide. (D) A plot of the intensities of the full amide peak area, the D-Ala amine peak area at 42 ppm, and the lysine peak area at 34 ppm as a function of the CP contact time for *S. aureus* MW2Δ*tarO* at OD 1.0. The MW2Δ*tarO* ^{15}N CP buildup and decay, including the slopes of the lines corresponding to long CP times, are the same as those of wild-type cells (Figure 4), allowing for direct comparisons between the MW2 wild-type and Δ*tarO* whole-cell spectra. All spectra are scaled by the total integrated spectral area. Each spectrum is the result of approximately 32,768 scans.