Electronic Supplementary Information for

Wall Teichoic Acids Govern Cationic Gold Nanoparticle Interaction with Gram-Positive Bacterial Cell Walls

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Contents

Results and Discussion

Solid-state ^{31}P-NMR analysis of sacculi. In both the solution and solid-state spectra for WTA from wild type bacteria (Fig. 4a), peak B is the most prominent and peak C is difficult to discern. Peak A is clearly resolved in the solution spectrum, is about half the height of peak B, and presents a small shoulder on its upfield side. Peak B exhibits a small shoulder on its downfield side. In the solid-state spectrum peak A broadened to the extent that it appears as a shoulder on peak B. In both the solution and solid-state spectra of sacculi isolated from the Δ*tagE* mutant (Fig. 4b), peak A is the most prominent, peak B is not discernible due to the lack of Glc substitution, and peak C appears between –0.5 and –1.5 ppm. The spectra for sacculi from the Δ*dltA* mutant resemble those of wild type (Fig. 4c). For both the solution and solid-state spectra, peak B is the most prominent, and peak C of Δ*dltA* is the most distinct among the three strains. In the solution spectrum, peak A is resolved, and both peaks A and B contain a small shoulder. In the solid-state spectrum, peak A presents as a shoulder downfield from peak B. Peak C is shifted upfield in the solid-state spectra for Δ*tagE* and Δ*dltA* strains, relative to their respective solution NMR spectra, likely due to the linkage of WTA to peptidoglycan in the sacculi samples.[1] We are unable to assess the direction of the shift in peak C for wild type due the difficulty in discerning this peak in the spectrum (Fig. 4a).

Experimental Methods

Materials. DNAse, RNAse, trypsin, chymotrypsin, and $CaCl_2$ used during sacculi isolation were used as received from Sigma-Aldrich (St Louis, MO). Gold nanoparticles (AuNPs, 10 nm diameter) functionalized with bPEI (BioPure) were purchased from nanoComposix (San Diego, CA). We selected 10 nm diameter gold nanoparticles for study because gold nanoparticles in this size range are commonly used in a variety of studies and products and were chosen to for benchmarking against other data. We obtained 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), 2-(*N*-morpholino) ethanesulfonic acid (MES), tris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl), cytochrome *c*, sodium dodecyl sulfate (SDS), hexamethylphosphoramide (99%), and branched polyethylenimine (M_w ~25 000) were purchased from Sigma-Aldrich (St Louis, MO). Marfey's reagent was procured from Thermo-Fischer Scientific (Waltham, MA).

Free bPEI polymer analysis. To quantify the amount of unbound bPEI, nanoparticle suspensions were sedimented by centrifugation (17,000g, 30 min), and the free bPEI in the supernatant was quantified by a fluorescamine assay.[2] The amount of free bPEI at a known concentration of bPEI-AuNPs was then determined using a calibration curve constructed with bPEI standards measured at λex/λem = 425 nm/480 nm.[3]

Bacterial viability analysis. The viability of bacteria exposed to bPEI-AuNPs or free bPEI was measured using a growth-based viability assay as previously described.[4] Using the growth conditions as described, the bacterial strains were suspended in HEPES buffer at a starting optical density (OD_{600}) of 0.05, which would represent 100% of viable bacteria. A calibration curve for

viable bacteria was obtained by serial diluting the initial culture with lower population of viable cells resulting in longer time to reach its exponential growth point. To assess toxicity of bPEI-AuNPs or free bPEI polymer, bacterial cells were incubated with nanoparticles for 15 min in HEPES buffer, and were introduced to fresh LB medium for growth. The toxicity induced by the particles decreased the initial viable cell population, and resulted in delays for the cultures to reach the exponential growth point. The percent of viable and actively metabolizing bacterial cells was then interpolated from the calibration curve.

Isolation of bacterial sacculi. Upon attainment of stationary phase, 1 mL of the suspension of cells grown on LB (1 mL) was transferred into 15 mL of fresh LB media in triplicate. This series of liquid inoculation was repeated once again for three times total. Two 15 mL aliquots were then combined to 500 mL of fresh LB broth. From this volume, the cultures were added to a 1 L baffled Erlenmeyer flask. Once the 1 L total of culture reached an OD_{600} of 0.6 (approximately 6 h), this solution became the initial starting solution for cell wall isolation for samples collected from LB media.

Once 1 L of culture was obtained for each strain in both LB and M9L media, flasks containing the cells were submerged in a 50:50 water:ethanol ice bath to rapidly cool the cell culture. Cells were harvested by centrifugation (7000*g*, 4 °C, 20 min). Sedimented cells were quantitatively transferred to a single 250 mL polypropylene centrifuge bottle (Thermo Scientific) containing 30 mL of ice cold 50 mM Tris-HCl, pH 7.4. To this was added 120 mL of hot 5% SDS to achieve a final concentration of 4% SDS, and the solution was placed in a boiling water bath for 30 min with the lid partially threaded on to allow pressure to escape as necessary. This solution then sat overnight at room temperature.[5,6]

Whole cells were recovered from the SDS solution through multiple ultracentrifugation steps.[7,8] Unless otherwise noted, all ultracentrifugation steps were performed at 46,000*g* for 30 min at 25 °C using an Optima L-90K Beckman Coulter, Inc. instrument. Cell suspensions (150 mL) were sedimented in 13.2 mL × 6 polypropylene ultracentrifuge tubes (14 × 89 mm capacity, Beckman Coulter). Pellets were washed twice with 1 M NaCl. To ensure pellet resuspension between washes, pellets were agitated with sterile, disposable inoculating loops and thorough mix pipetting. Each washing step required a 30 min ultracentrifugation spin step to sediment the pellets. The resulting six pellets were combined into three via quantitative transfer. Pellets went through four cycles of washing with ultrapure water to remove SDS, agitation, and sedimentation, using the method described by Hayashi.[9] After these washing steps, 1 mL of ultrapure water was added to the pellets, and they were allowed to sit overnight.

The following day, the cells were agitated and re-suspended using sterile inoculating loops. The solution was divided into two parts and each 500 μL aliquot was transferred to a vial containing 350 μL of 100 μm acid washed glass beads (2.0 mL capacity, Benchmark Scientific). The cells were mechanically lysed using a FastPrep-24 instrument for 15 rounds using a setting of 6.5 $m \cdot s^{-1}$ for 20 s with 1 min rest intervals between the pulses. To separate the beads from the lysed cells, bead beating vials were washed with ~30 mL of ultrapure water to ensure complete sample

removal into a 50 mL Falcon polypropylene centrifuge tube and centrifuged (3000*g*, 2 min) using a Rotanta 460 RS centrifuge (Hettich Zentrifugen). Glass beads were sedimented, leaving the bacterial suspension in the supernatant. This method was reliable yet unsatisfactory as it left the margin for glass bead contamination in the sample. This technique was used for cell wall isolation from LB broth, yet a second method was developed for separating the cells from the beads for the cell walls isolated from M9L media. Rather than centrifuging at 3000*g*, a vacuum filter system with a rough fritted glass filter (pore size of 45-60 μm) was used. Because lysed bacterial cells are significantly smaller than this pore size, only glass beads were retained in the filter. A drawback to both methods is that they yielded large volumes of supernatant or filtrate such that multiple steps were required to condense lysed cells into a single ultracentrifuge tube.

Cell remnants were suspended in 10 mL of enzyme buffer (100 mM Tris-HCl, 20 mM $MgSO_4$, pH 7.5). Nucleic acid digestion was performed using 10 $\mu g \cdot mL^{-1}$ DNAse and 50 $\mu g \cdot mL^{-1}$ RNAse at 37 °C for 2 h. Proteins were digested by adding 10 mM $CaCl_2$, trypsin and chymotrypsin (both at 100 $\mu g \cdot mL^{-1}$). Proteolysis was allowed to proceed for 18 h in a shaker at 37 °C and 150 rpm. Digestion was halted by addition of 1% SDS and incubation for 15 min at 80 °C in a water bath (Dubnoff Metabolic Shaking Incubator, Precision Thermo Electron Corporation). Crude cell wall was recovered by ultracentrifugation, re-suspended in 8 M LiCl, and incubated for 15 min at 37 °C. The pellet was washed in the following sequence: twice with ultrapure water, once with acetone, and four times with ultrapure water. Samples were stored at –80 °C until lyophilization (24 h, –50 °C, 60 mTorr).[10] This yielded a white to light yellow semi-crystalline powder. Total sacculi yields varied with bacterial strain and growth medium (roughly 100 mg of lyophilized sacculi per strain and growth medium). Solution ^{31}P-NMR was also used to quantify the amount of phosphorus in WTA isolates (*vide infra*).

Characterization of D-alanine in WTA. To verify that the WTA produced by the Δ*dltA* mutant lacked D-alanine, we quantified the amount of alanine in isolated wall teichoic acid using reverse-phase HPLC with UV detection. Bacterial cells were sedimented by centrifugation (1,000*g*, 10 min) and deactivated by heating (100 °C, 30 min). The sample pellets were frozen (–80 °C, 24 h) and lyophilized (Labconco FreeZone 1 Liter Benchtop Freeze Dry System, Kansas City, MO). An aliquot of lyophilized sample (5 mg) was resuspended in 150 μL of 0.1 M NaOH and incubated for 37 °C for 1 h, followed by neutralization with 150 μL of 0.1 M HCl. Samples were centrifuged (750*g*, 10 min), and the supernatant containing hydrolyzed alanine was removed, frozen (–80 °C, 24 h), and lyophilized. Lyophilized WTA was suspended in 100 μL of ultrapure water, reacted with 200 μL of 1% Marfey's reagent in acetone and 40 μL of 1 M sodium bicarbonate at 40 °C for 1 h on a heating block. Once the reaction was complete, samples were neutralized with 40 μL of 1 M HCl, degassed, separated via HPLC using a C–18 column (Agilent Eclipse-XDB, 4.6x150 mm), and quantified based on absorbance at 340 nm. Gradient elution of mobile phase was carried out from 0-50% acetonitrile in sodium acetate buffer over 10 min, followed by isocratic elution at 50% of acetonitrile for 3 min.[11]

Table S1 Solid-state ^{31}P-NMR peak shifts (ppm) of sacculi from wild type, Δ*tagE*, Δ*dltA* strains of *Bacillus subtilis*.

	wild type	Δ*tagE*	Δ*dltA*
Peak A	0.90	0.77	0.62
Peak B	0.56	n/a	0.37
Peak C	n/a*	–1.17	–1.20

*Specific peak shift unobtainable due to peak broadening.

Table S2 Solid-state ^{31}P-NMR chemical shifts and widths of sacculi from wild type (peak B), *ΔtagE* (peak A), *ΔdltA* (peak B) strains of *Bacillus subtilis*, and chemical shift change and direction of sacculi with bPEI-AuNPs and free bPEI polymer. Peaks tabulated correspond with the dominant peak in the respective spectra.

	sacculi		+ bPEI-AuNPs				+ free bPEI polymer			
strain	peak shift (ppm)	peak width (Hz)	peak shift (ppm)	peak width (Hz)	Δ peak shift (ppm)	Δ direction	peak shift (ppm)	peak width (Hz)	Δ peak shift (ppm)	Δ direction
wild type	0.56	49.51	0.39	103.26	–0.17	upfield	1.85	83.76	+1.29	downfield
Δ*tagE*	0.77	48.75	0.63	63.26	–0.14	upfield	2.19	61.52	+1.42	downfield
Δ*dltA*	0.37	62.6	0.51	77.74	+0.14	downfield	2.20	66.59	+1.83	downfield

Table S3 Solid-state ^{13}C-NMR chemical shifts (ppm) of sacculi from wild type, Δ*tagE*, Δ*dltA* strains of *Bacillus subtilis*.

		wild type	Δ*tagE*	Δ*dltA*
	peptidoglycan			
GlcNAc				
	C1	100.73	100.99	100.69
	C2	42.24	42.32	42.26
	C3	67.91	67.93	67.90
	C4	90.99	90.77	90.92
	C5	72.28	72.90	72.11
	C6	50.34	50.50	50.09
	NHAc CO	169.56	169.6	169.55
	NHAc CH_3	20.97	21.04	20.98
MurNAc				
	C1	101.39	101.97	101.87
	C2	42.24	42.32	42.26
	C3	90.17	89.03	90.11
	C4	90.99	90.08	90.92
	C5	67.91	67.93	67.90
	C6	50.34	50.50	50.09
	NHAc CO	169.56	169.6	169.55
	NHAc CH_3	20.97	21.04	20.98
D-Lac				
	CO	169.56	169.6	169.55
	Cα	89.31	88.34	89.60
	CH_3	17.92	18.05	17.77
L-Ala and D-Ala				
	Cα	37.92	39.09	38.21
	Cβ	17.92	18.05	17.77
	CO	169.56	169.60	169.55
γ- D-Glu				
	Cα	40.55	41.42	40.40
	Cβ	22.84	23.02	22.75
	Cγ	31.77	31.88	31.90

	CO_2^-	169.56	169.6	169.55
	CONH	169.56	169.6	169.55
meso-A_2pm				
	Cα	39.38	40.55	39.53
	Cβ	28.83	29.02	28.72
	Cγ	19.67	19.67	19.52
	Cδ	19.67	19.67	19.52
	Cϵ	19.67	19.67	19.52
	CO	169.56	169.6	169.55
wall teichoic acids				
glycerolphosphate	C1	55.06	55.31	55.14
	C2	75.77	75.44	75.51
	C3	55.06	55.31	55.14
GlcNAc and ManNAc				
	C1	100.14	99.49	99.97
	C2	42.24	42.32	42.26
	C3	79.49	79.63	79.2
	C4	60.80	60.95	60.84
	C5	65.95	67.12	65.81
	C6	50.34	50.50	50.09
	NHAc CO	169.56	169.6	169.55
	NHAc CH_3	20.97	21.04	20.98
Glc				
	C1	99.28[†]	n/a	98.00[†]
	C2	74.13[†]	n/a	74.33[†]
	C3	74.13[†]	n/a	74.33[†]
	C4	70.13[†]	n/a	70.04[†]
	C5	74.13[†]	n/a	74.33[†]
	C6	61.85[†]	n/a	62.20[†]

[†] Signal-to-noise low, peak assignment tentative.

Abbreviations: GlcNAc, *N*-acetylglucosamine; ManNAc, *N*-acetylmannosamine; MurNAc, *N*-acetylmuramic acid; D-Ala, D-alanine; L-Ala, L-alanyl; *meso*-A_2pm, *meso*-diaminopimelyl; Glc, glucose; D-γ-Glu, D-γ-glutamyl; D-Lac, D-lactyl

Table S4 Linear fit equations of temperature-dependent T_1 relaxation times for specified ^{13}C resonances of sacculi from wild type, Δ*tagE*, Δ*dltA* strains of *Bacillus subtilis*. In peptidoglycan residues, C3 refers to GlcNAc, and C5 is of MurNAc.

	wild type	**Δ*tagE***	**Δ*dltA***
peptidoglycan			
C3, C5	T_1 = -0.041 (± 0.0091)T + 3.6 (± 0.15)†	T_1 = -0.025 (± 0.0037)T + 3.3 (± 0.063)†	T_1 = -0.030 (± 0.0028)T + 3.9 (± 0.048)†
C4	T_1 = -0.070 (± 0.015)T + 3.2 (± 0.26)†	T_1 = -0.076 (± 0.016)T + 4.0 (± 0.28)†	T_1 = -0.050 (± 0.0096)T + 3.5 (± 0.16)†
Glu Cγ	T_1 = -0.0079 (± 0.0021)T + 0.76 (± 0.037)†	T_1 = -0.0037 (± 0.0012)T + 0.63 (± 0.020)	T_1 = -0.0051 (± 0.00086)T + 0.72 (± 0.015)†
meso-A_2pm Cβ	T_1 = -0.016 (± 0.0040)T + 0.89 (± 0.069)†	T_1 = -0.011 (± 0.0016)T + 0.67 (± 0.028)†	T_1 = -0.0063 (± 0.00034)T + 0.68 (± 0.0058)†
wall teichoic acids			
C1, C3	T_1 = -0.010 (± 0.0017)T + 0.67 (± 0.029)†	T_1 = -0.0089 (± 0.0026)T + 0.51 (± 0.045)†	T_1 = -0.0092(± 0.0028)T + 0.64 (± 0.048)†
C2	T_1 = -0.011 (± 0.00087)T + 0.74 (± 0.015)†	T_1 = -0.014 (± 0.00084)T + 0.83 (± 0.014)†	T_1 = -0.023 (± 0.0031)T + 1.1 (± 0.054)†

† Significant non-zero linear fit.

Abbreviations: T_1, longitudinal relaxation time; T, temperature.

Table S5 Solid-state ^{13}C-NMR chemical shifts (ppm) of sacculi from wild type strain of *Bacillus subtilis*, with bPEI-AuNPs and free bPEI polymer.

		wild type		
		sacculi	+ bPEI AuNPs	+ free bPEI polymer
peptidoglycan				
GlcNAc				
	C1	100.73	100.83	100.96
	C2	42.24	42.58	42.57
	C3	67.91	68.22	68.18
	C4	90.99	–	–
	C5	72.28	–	–
	C6	50.34	50.47[§]	–
	NHAc CO	169.56	169.78	169.81
	NHAc CH_3	20.97	21.27	21.24
MurNAc				
	C1	101.39	101.26[§]	–
	C2	42.24	42.58	42.57
	C3	90.17	90.30	90.32
	C4	90.99	–	–
	C5	67.91	68.22	68.18
	C6	50.34	50.47[§]	–
	NHAc CO	169.56	169.78	169.81
	NHAc CH_3	20.97	21.27	21.24
D-Lac				
	CO	169.56	169.78	169.81
	Cα	89.31	–	–
	CH_3	17.92	18.20[§]	–
L-Ala and D-Ala				
	Cα	37.92	38.20	38.13
	Cβ	17.92	18.20[§]	–
	CO	169.56	169.78	169.81
γ- D-Glu				
	Cα	40.55	40.98	40.91
	Cβ	22.84	23.02	22.80[§]

	Cγ	31.77	32.58	32.76[§]
	CO_2^-	169.56	169.78	169.81
	CONH	169.56	169.78	169.81
meso-A_2pm				
	Cα	39.38	39.66	39.59
	Cβ	28.83	29.07	29.28[§]
	Cγ	19.67	19.95	19.74
	Cδ	19.67	19.95	19.74
	Cϵ	19.67	19.95	19.74
	CO	169.56	169.78	169.81
wall teichoic acids				
glycerolphosphate	C1	55.06	55.73	–
	C2	75.77	–	–
	C3	55.06	55.73	–
GlcNAc and ManNAc				
	C1	100.14	–	100.14[§]
	C2	42.24	42.58	42.57
	C3	79.49	–	–
	C4	60.80	60.66	–
	C5	65.95	–	–
	C6	50.34	51.04	–
	NHAc CO	169.56	169.78	169.81
	NHAc CH_3	20.97	21.27	21.24
Glc				
	C1	99.28[†]	–	–
	C2	74.13[†]	–	–
	C3	74.13[†]	–	–
	C4	70.13[†]	–	–
	C5	74.13[†]	–	–
	C6	61.85[†]	61.77[†]	–

Notation: –, no detectable peak; [†], peak assignment tentative; [§], low signal-to-noise ratio.
Abbreviations: GlcNAc, *N*-acetylglucosamine; ManNAc, *N*-acetylmannosamine; MurNAc, *N*-acetylmuramic acid; D-Ala, D-alanine; L-Ala, L-alanyl; *meso*-A_2pm, *meso*-diaminopimelyl; Glc, glucose; D-γ-Glu, D-γ-glutamyl; D-Lac, D-lactyl.

Table S6 Solid-state ^{13}C-NMR chemical shifts (ppm) of sacculi from Δ*tagE* strain of *Bacillus subtilis*, with bPEI-AuNPs and free bPEI polymer.

		Δ*tagE*		
		sacculi	+ bPEI AuNPs	+ free bPEI polymer
peptidoglycan				
GlcNAc				
	C1	100.99	101.34	101.24
	C2	42.32	42.92	42.63
	C3	67.93	68.58	68.22
	C4	90.77	91.35	91.57
	C5	72.90	73.70	72.97
	C6	50.50	51.02	49.84
	NHAc CO	169.60	170.28	169.85
	NHAc CH_3	21.04	21.65	21.25
MurNAc				
	C1	101.97	–	–
	C2	42.32	42.92	42.63
	C3	89.03	90.03	–
	C4	90.08	90.71	90.23
	C5	67.93	68.58	68.22
	C6	50.50	51.02	49.84
	NHAc CO	169.60	170.28	169.85
	NHAc CH_3	21.04	21.65	21.25
D-Lac				
	CO	169.60	170.28	169.85
	Cα	88.34	89.20	88.36
	CH_3	18.05	18.64	18.11
L-Ala and D-Ala				
	Cα	39.09	39.22	39.13
	Cβ	18.05	18.64	18.11
	CO	169.60	170.28	169.85
γ- D-Glu				
	Cα	41.42	41.56	–
	Cβ	23.02	23.44	23.10

	Cγ	31.88	32.86	31.49
	CO_2^-	169.60	170.28	169.85
	CONH	169.60	170.28	169.85
meso-A_2pm				
	Cα	40.55	40.54	40.30
	Cβ	29.02	29.91	29.09
	Cγ	19.67	20.53	20.01
	Cδ	19.67	20.53	20.01
	Cϵ	19.67	20.53	20.01
	CO	169.60	170.28	169.85
wall teichoic acids				
glycerolphosphate	C1	55.31	56.11	55.40
	C2	75.44	76.02	75.37
	C3	55.31	56.11	55.40
GlcNAc and ManNAc				
	C1	99.49	100.54[§]	–
	C2	42.32	42.92	42.63
	C3	79.63	–	80.08[§]
	C4	60.95	61.48	61.12
	C5	67.12	66.82	–
	C6	50.50	51.02	49.84
	NHAc CO	169.60	170.28	169.85
	NHAc CH_3	21.04	21.65	21.25
Glc				
	C1 – C6		n/a	

Notation: –, no detectable peak; [§], low signal-to-noise ratio.

Abbreviations: GlcNAc, *N*-acetylglucosamine; ManNAc, *N*-acetylmannosamine; MurNAc, *N*-acetylmuramic acid; D-Ala, D-alanine; L-Ala, L-alanyl; *meso*-A_2pm, *meso*-diaminopimelyl; Glc, glucose; D-γ-Glu, D-γ-glutamyl; D-Lac, D-lactyl.

Table S7 Solid-state ^{13}C-NMR chemical shifts (ppm) of sacculi from Δ*dltA* strain of *Bacillus subtilis*, with bPEI-AuNPs and free bPEI polymer.

		Δ*dltA*		
		sacculi	+ bPEI AuNPs	+ free bPEI polymer
peptidoglycan				
GlcNAc				
	C1	100.69	100.82	101.04
	C2	42.26	42.40	42.56
	C3	67.90	68.08	68.17
	C4	90.92	90.91	91.22
	C5	72.11	72.74	72.37
	C6	50.09	50.46	50.65
	NHAc CO	169.55	169.67	169.80
	NHAc CH_3	20.98	21.14	21.23
MurNAc				
	C1	101.87	102.15	102.02
	C2	42.26	42.40	42.56
	C3	90.11	90.27	90.24
	C4	90.92	90.91	91.22
	C5	67.90	68.08	68.17
	C6	50.09	50.46	50.65
	NHAc CO	169.55	169.67	169.80
	NHAc CH_3	20.98	21.14	21.23
D-Lac				
	CO	169.55	169.67	169.80
	Cα	89.60	89.59	89.35
	CH_3	17.77	18.12	18.39
L-Ala and D-Ala				
	Cα	38.21	39.81	39.34
	Cβ	17.77	18.12	18.39
	CO	169.55	169.67	169.80
γ- D-Glu				
	Cα	40.40	41.56	40.73
	Cβ	22.75	22.96	23.22

	Cγ	31.90	31.21	31.36
	CO_2^-	169.55	169.67	169.80
	CONH	169.55	169.67	169.80
meso-A_2pm				
	Cα	39.53	40.68	40.14
	Cβ	28.72	29.04	29.32
	Cγ	19.52	19.80	20.00
	Cδ	19.52	19.80	20.00
	Cϵ	19.52	19.80	20.00
	CO	169.55	169.67	169.80
wall teichoic acids				
glycerolphosphate	C1	55.14	55.43	55.47
	C2	75.51	75.71	75.55
	C3	55.14	55.43	55.47
GlcNAc and ManNAc				
	C1	99.97	98.35	99.91
	C2	42.26	42.40	42.56
	C3	79.20	78.82	78.60
	C4	60.84	60.96	60.94
	C5	65.81	66.52	66.86
	C6	50.09	50.46	50.65
	NHAc CO	169.55	169.67	169.80
	NHAc CH_3	20.98	21.14	21.23
Glc				
	C1	98.00†	97.44†	98.39†
	C2	74.33†	–	74.56†
	C3	74.33†	–	74.56†
	C4	70.04†	70.03†	–
	C5	74.33†	–	74.56†
	C6	62.20†	62.39†	62.32†

Notation: –, no detectable peak; †, signal-to-noise low, peak assignment tentative.
Abbreviations: GlcNAc, *N*-acetylglucosamine; ManNAc, *N*-acetylmannosamine; MurNAc, *N*-acetylmuramic acid; D-Ala, D-alanine; L-Ala, L-alanyl; *meso*-A_2pm, *meso*-diaminopimelyl; Glc, glucose; D-γ-Glu, D-γ-glutamyl; D-Lac, D-lactyl.

Table S8 Minimal growth medium formula.[12]

base solution	component	concentration [mM]
	$Na_2HPO4•7H_2O$	37.3
	KH_2PO_4	22
	NaCl	8.5
	$^{15}NH_4Cl$	18.6
supplements	**component**	**concentration [mM]**
	^{13}C-glucose (all C labeled)	22
	$MgSO_4$	1
	$CaCl_2$	0.1
	$MnCl_2$	0.1
	$ZnSO_4$	0.05
vitamins	**component**	**concentration [µM]**
	pyridoxin	295
	biotin	205
	panthothenate, hemi calcium	228
	folic acid	113
	choline chloride	358
	niacin amide	409
	riboflavin	133
	thiamine	741

Table S9 Composition of Luria-Bertani (LB) medium.

component	concentration (g/L)
tryptone	10
yeast extract	5
NaCl	10

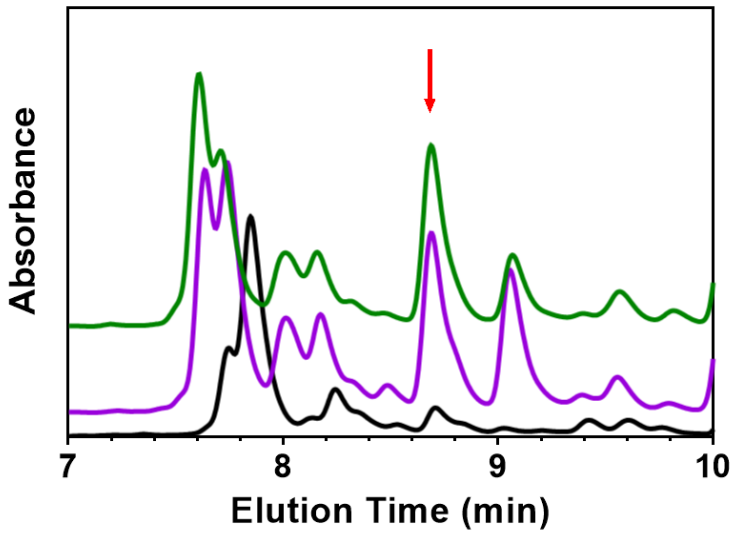


Fig. S1 HPLC-UV chromatogram of hydrolyzed WTA material from the wild type (green), Δ*tagE* mutant (purple), and Δ*dltA* mutant (black) of *Bacillus subtilis.* The red arrow indicates the retention time of derivatized alanine eluting at 8.7 min. The experimental procedure is described in the ESI.

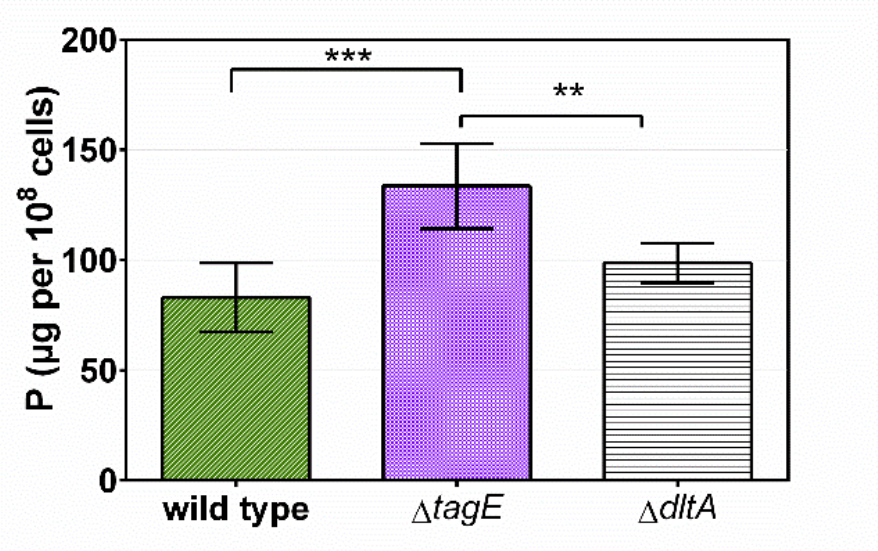


Fig. S2 Phosphate content in isolated wall teichoic acid chains per 10^8 *B. subtilis* cells, as estimated from OD measurements (**, $p < 0.01$; ***, $p < 0.005$). The amounts of WTA analyzed for each strain were from cell suspensions normalized with equivalent optical density. The amount of phosphate in WTA was determined using an ascorbic acid-ammonium molybdate solution that forms a blue complex in the presence of inorganic phosphate. The amount of phosphate in the isolated WTA of Δ*tagE* mutant was higher than that of wild type *B. subtilis* and the Δ*dltA* mutant ($p < 0.05$), in agreement with the ^{31}P-NMR spectra (Fig. 3).

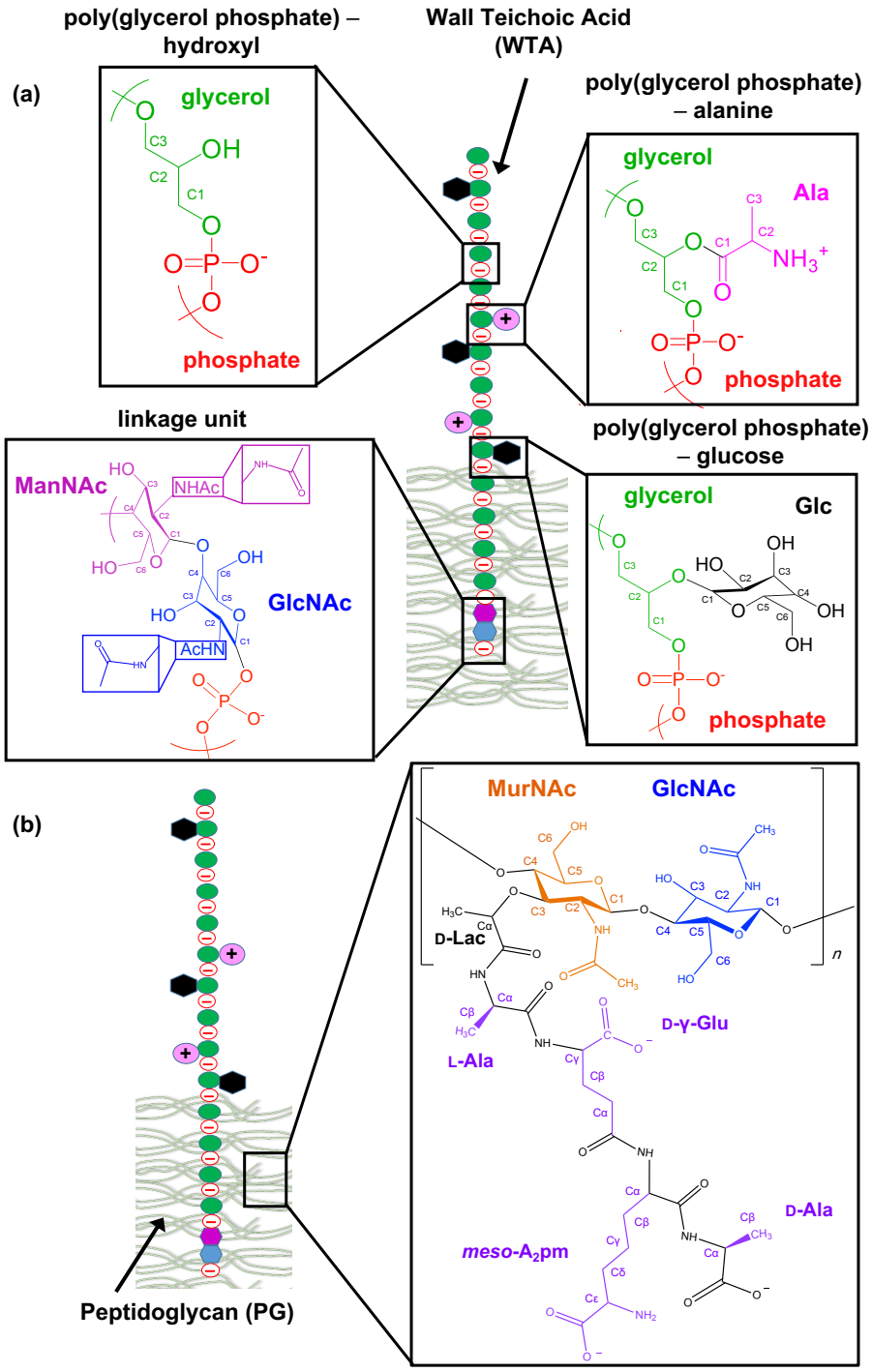


Fig. S3 Labeled carbon atoms in (a) wall teichoic acids (linker unit and glycerolphosphate with side groups (–OH, –alanine, and –glucose) and (b) peptidoglycan (glycans and peptide). Note that alanine is removed during the sacculi isolation process, and therefore does not appear in ^{13}C-NMR spectra. Abbreviations: GlcNAc, *N*-acetylglucosamine; ManNAc, *N*-acetylmannosamine; MurNAc, *N*-acetylmuramic acid; D-Ala, D-alanine; L-Ala, L-alanyl; *meso*-A2pm, *meso*-diaminopimelyl; Glc, glucose; D-γ-Glu, D-γ-glutamyl; D-Lac, D-lactyl.

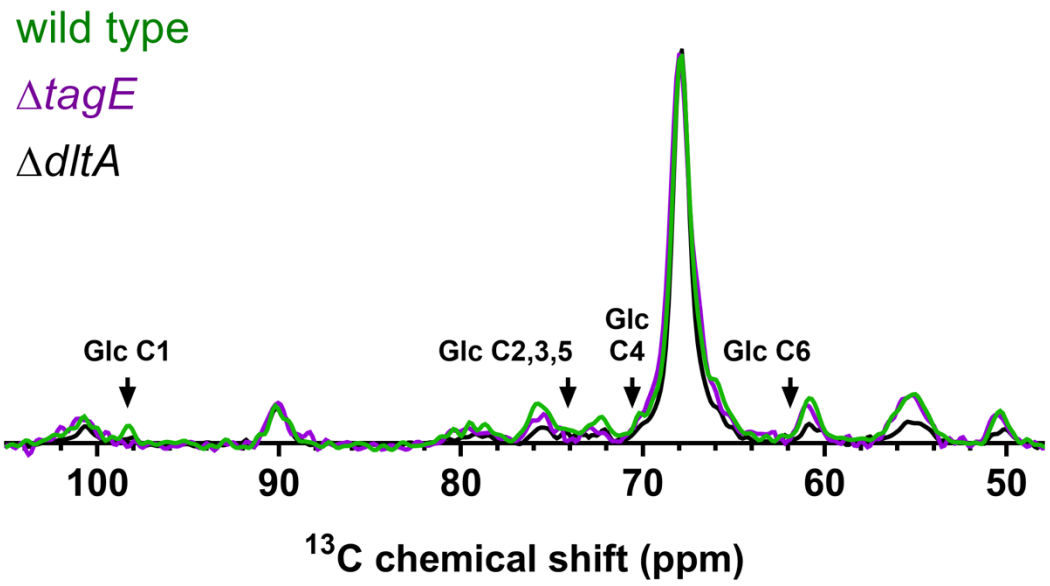


Fig. S4 Expanded region of solid-state ^{13}C-NMR spectra of sacculi from wild type (green), Δ*tagE* (purple), and Δ*dltA* (black) displaying peaks tentatively assigned to Glc carbons (C1-C6) for Δ*dltA* for WTA strains.

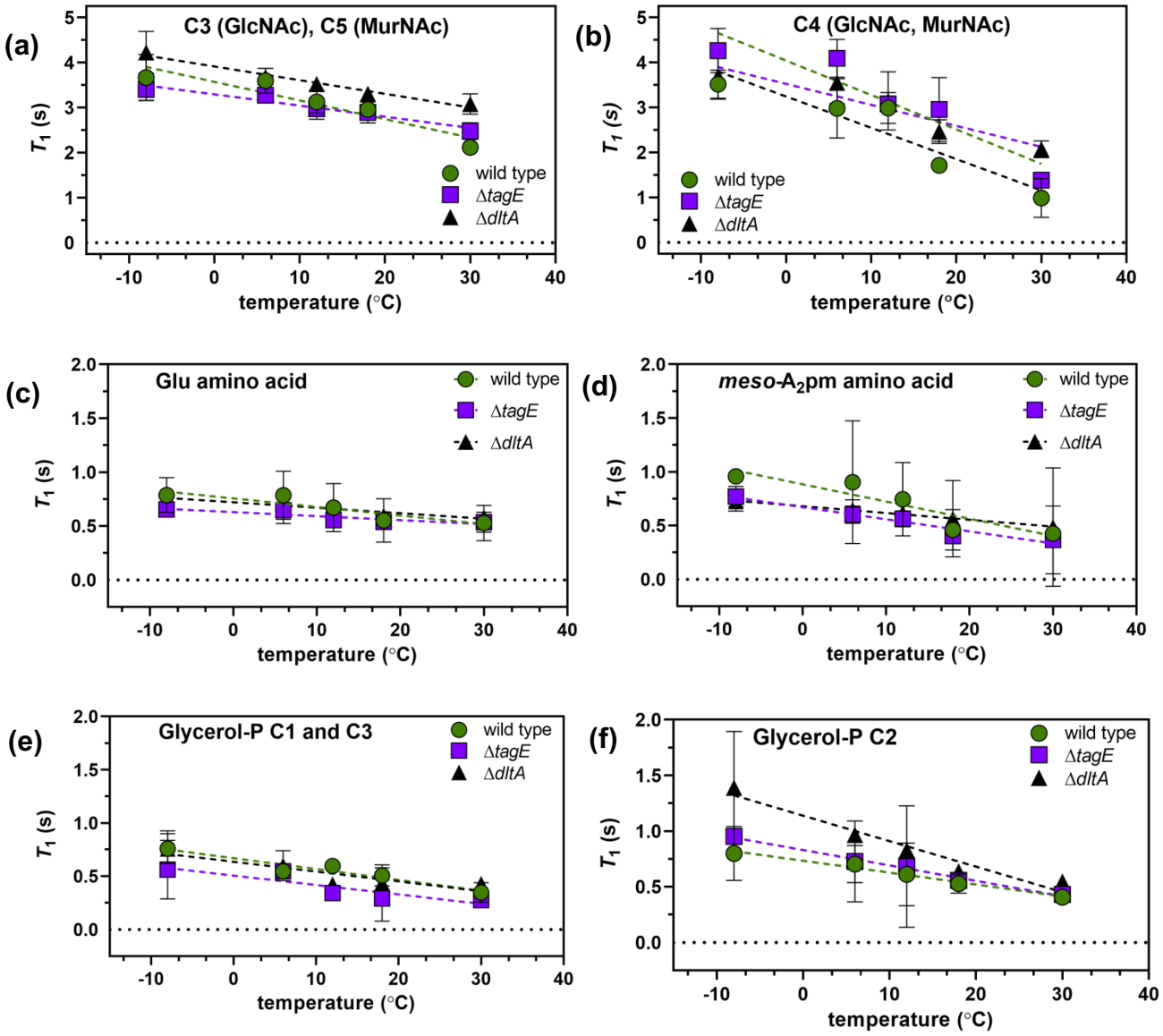
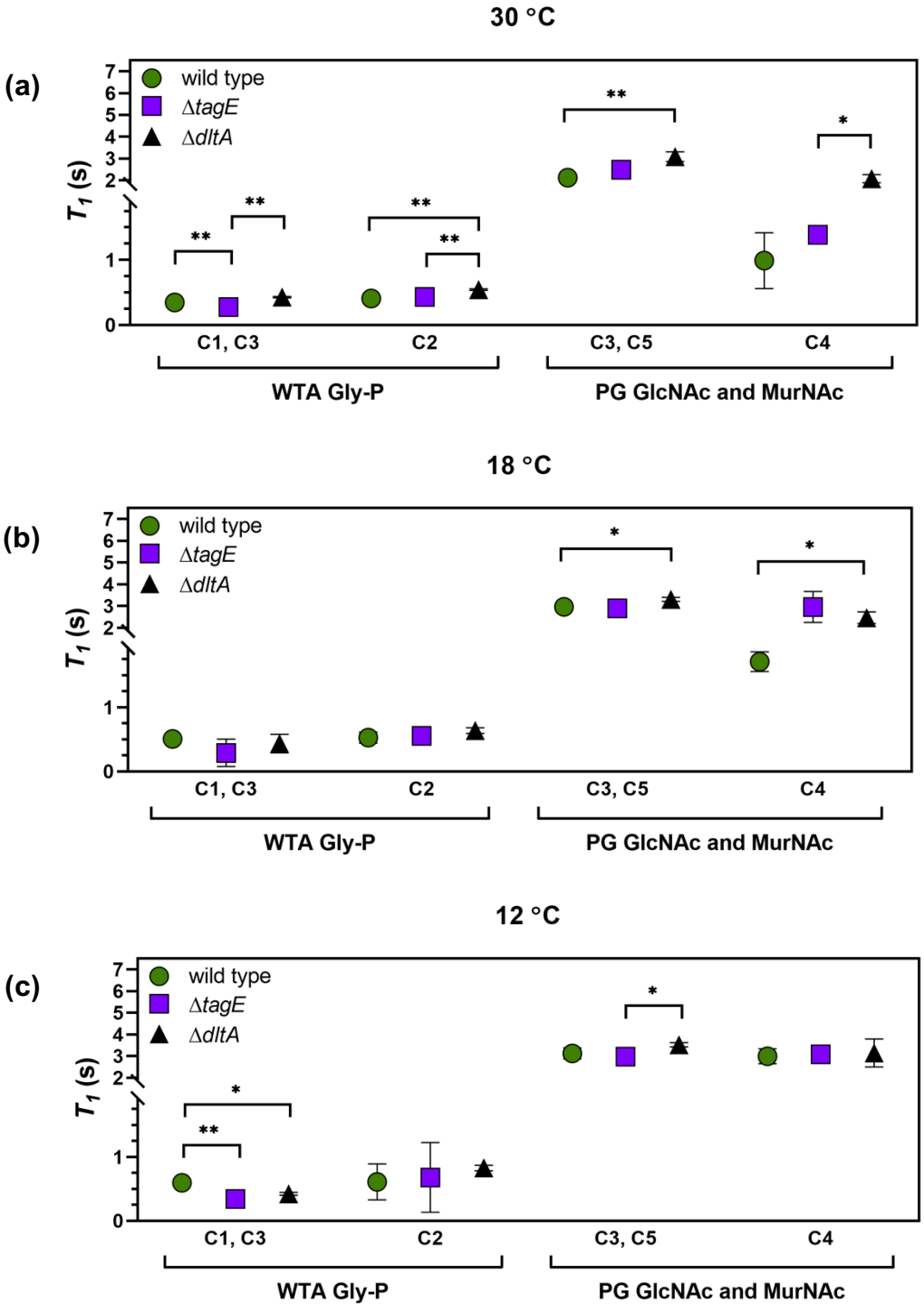


Fig. S5 Linear fits of temperature-dependent T_1 relaxation times for specified 13C resonances of sacculi from wild type, Δ*tagE*, Δ*dltA* strains of *Bacillus subtilis*: (a) GlcNAc C3 and MurNAc C5 of peptidoglycan, (b) GlcNAc and MurNAc C4 of peptidoglycan, (c) Glu Cγ of peptidoglycan, (d) *meso*-A_2pm Cβ of peptidoglycan, (e) glycerolphosphate C1 and C3 of wall teichoic acids, and (f) glycerolphosphate C2 of wall teichoic acids. Error bars represent standard error in non-linear fitting from T_1 relaxation time equation.


30 °C
(a)
wild type
ΔtagE
ΔdltA
T1 (s)
C1, C3
C2
C3, C5
C4
WTA Gly-P
PG GlcNAc and MurNAc
18 °C
(b)
12 °C
(c)

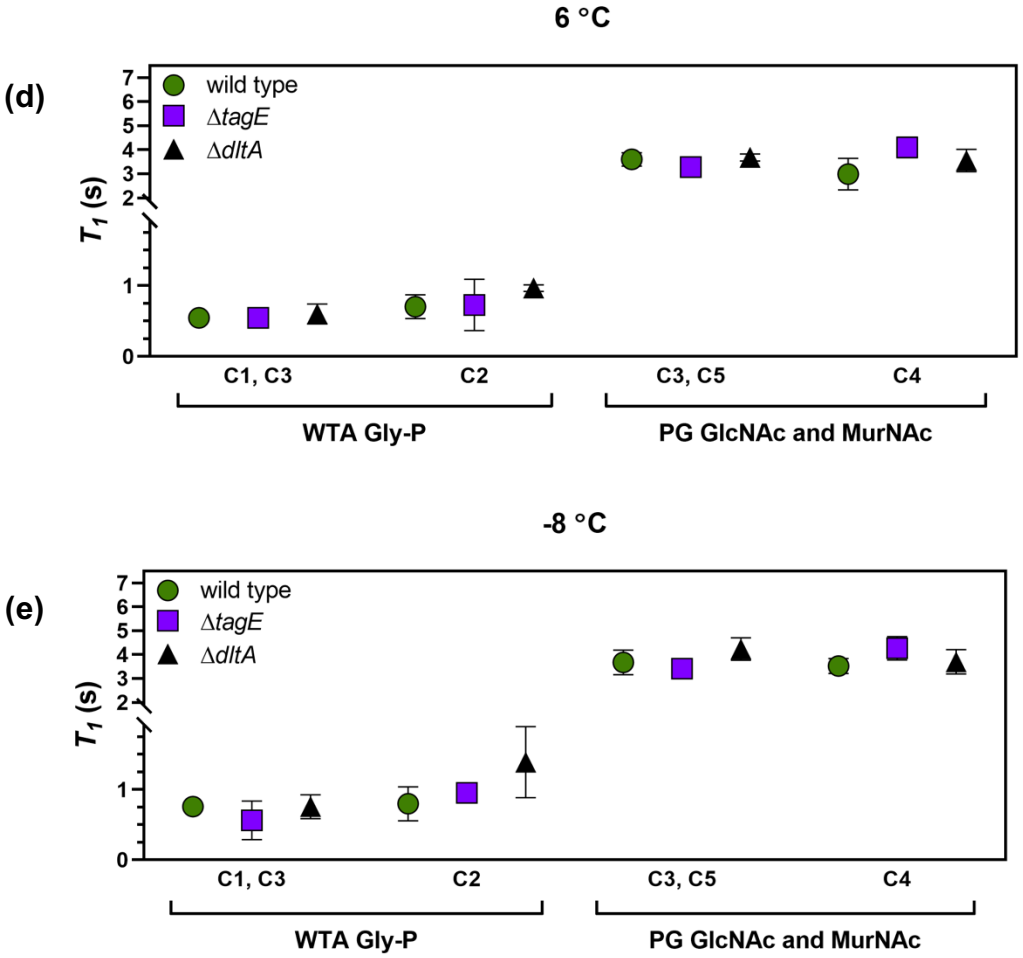


Fig. S6 T_1 relaxation times at indicated temperature (a) 30 °C, (b) 18 °C, (c) 12 °C, (d) 6 °C, (e) –8 °C for specified ^{13}C resonances of sacculi from wild type, Δ*tagE*, Δ*dltA* strains of *Bacillus subtilis*: GlcNAc C3 and MurNAc C5 of peptidoglycan, GlcNAc and MurNAc C4 of peptidoglycan, glycerolphosphate C1 and C3 of wall teichoic acids, and glycerolphosphate C2 of wall teichoic acids. T_1 relaxation times of Glu Cγ and *meso*-A_2pm Cβ amino acids of peptidoglycan showed no statistically significant differences among the three strains at any of the five temperatures (data not shown). Non-linear fit statistical significance: *, $p < 0.05$; **, $p < 0.01$. Error bars represent standard error in non-linear fitting from T_1 relaxation time equation. Abbreviations: GlcNAc, *N*-acetylglucosamine; MurNAc, *N*-acetylmuramic acid.

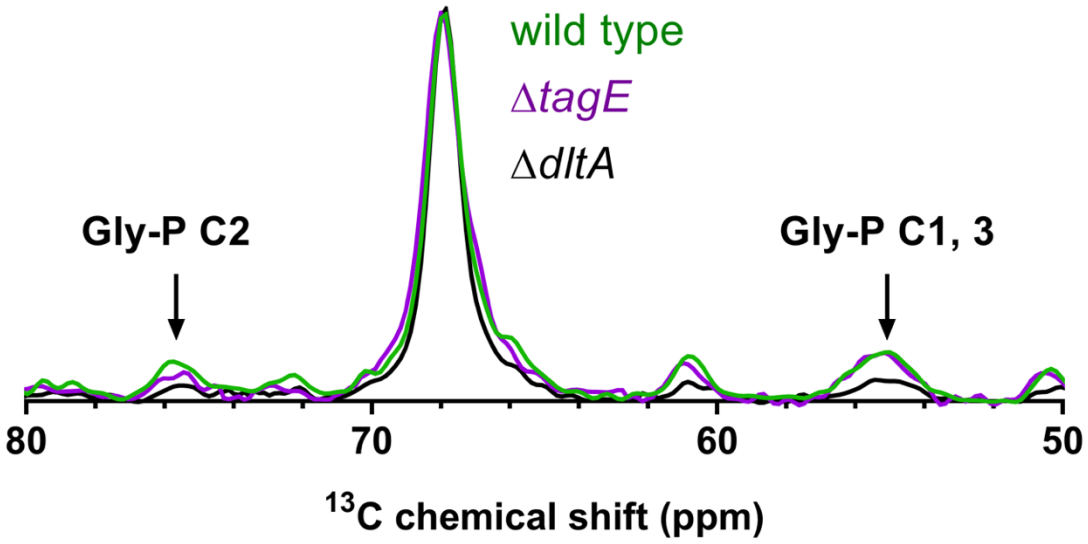


Fig. S7 Expanded region of solid-state ^{13}C-NMR spectra of sacculi from wild type (green), Δ*tagE* (purple), and Δ*dltA* (black) showing smaller peak areas for Δ*dltA* for WTA glycerolphosphate carbon peaks (C1, C2, C3). The smaller peak areas in Δ*dltA* reflect the presence of fewer glycerolphosphate carbons relative to the other strains or peak broadening due to their close proximity to peptidoglycan molecules.

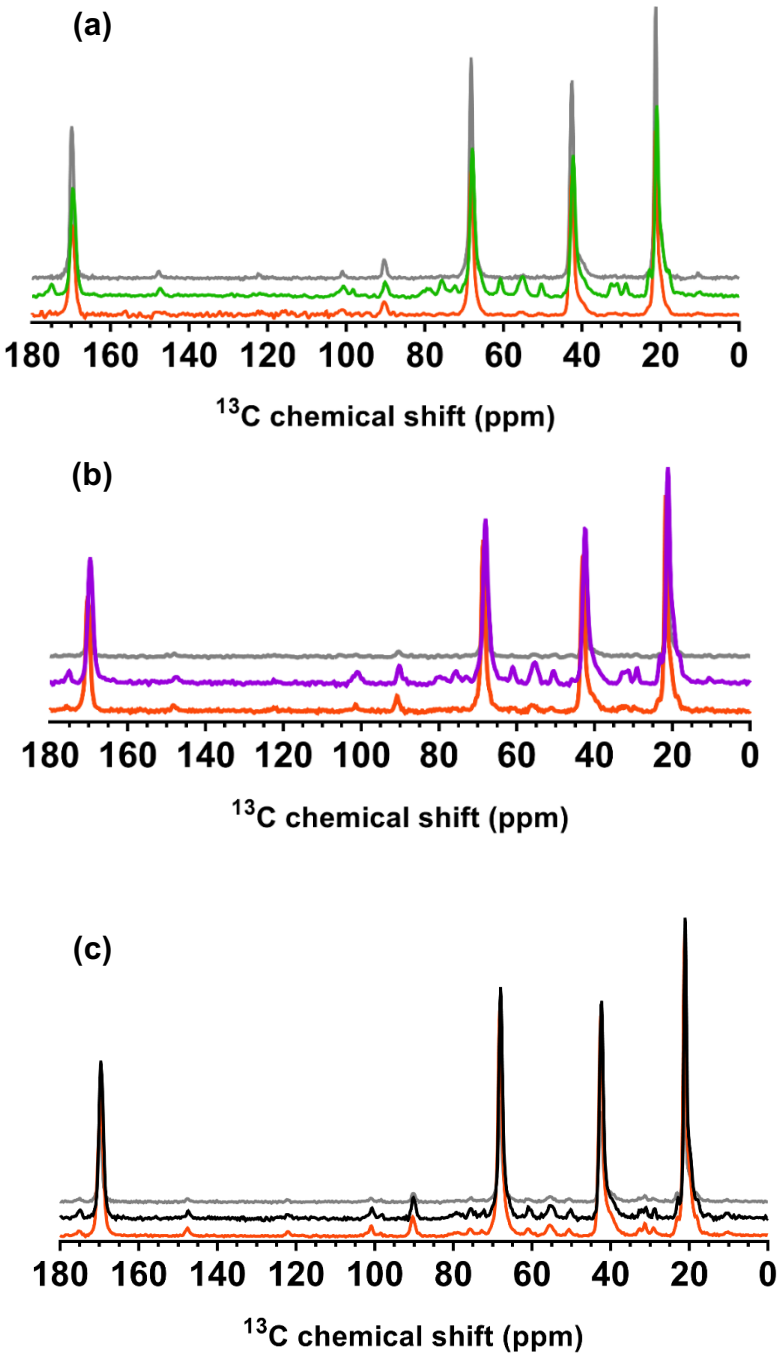


Fig. S8 Solid-state ^{13}C-NMR spectra of sacculi from (a) wild type, (b) Δ*tagE*, and (c) Δ*dltA* where the orange trace shows the result of 34 nM bPEI-AuNPs and the grey trace shows the impact 29.1 μM free bPEI polymer**.**

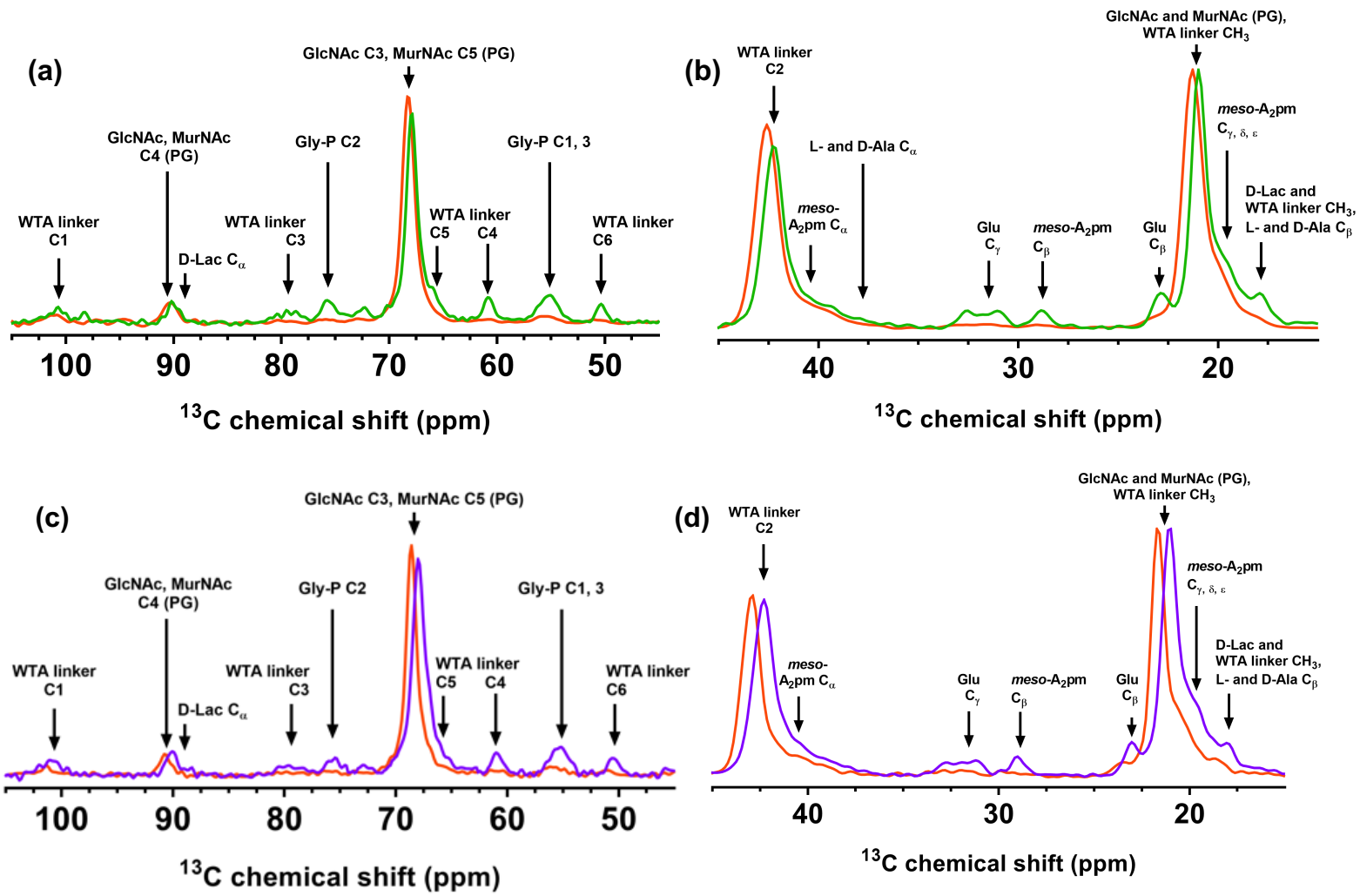


Fig. S9 Expanded regions of solid-state ^{13}C-NMR spectra of sacculi from (a, b) wild type, green traces, and (c, d) Δ*tagE*, purple traces, with the addition of 34 nM bPEI-AuNPs (orange traces). Spectral regions display peak broadening or slight shifts for the corresponding labeled carbon residues. Abbreviations: GlcNAc, *N*-acetylglucosamine; ManNAc, *N*-acetylmannosamine; MurNAc, *N*-acetylmuramic

acid; D-Ala, D-alanine; L-Ala, L-alanyl; *meso*-A_2pm, *meso*-diaminopimelyl; Glc, glucose; D-γ-Glu, D-γ-glutamyl; D-Lac, D-lactyl. WTA linker unit is composed of GlcNAc and ManNAc amino sugars.

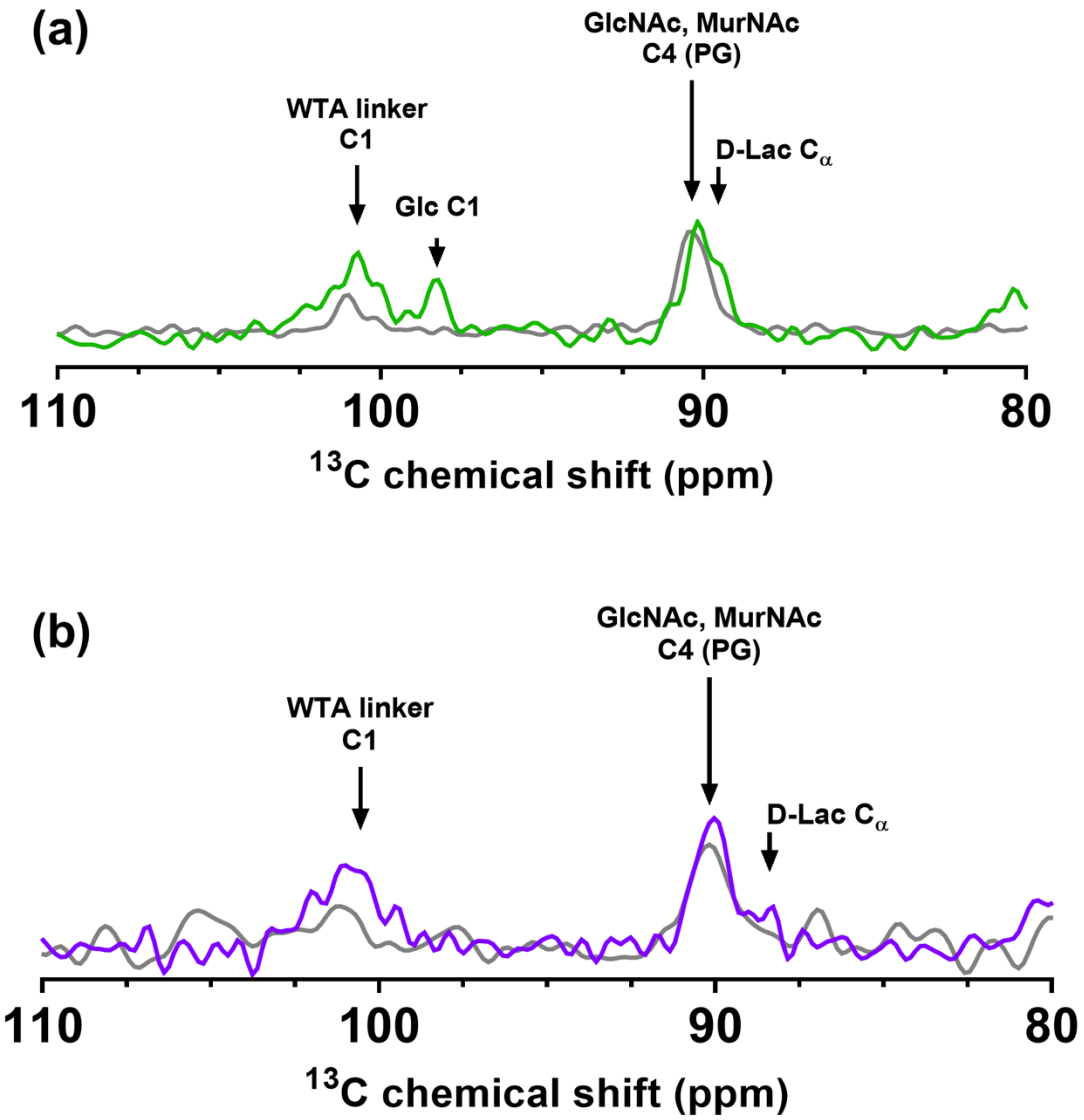


Fig. S10 Expanded regions of solid-state ^{13}C-NMR spectra of sacculi from (a,) wild type, green trace, and (b) Δ*tagE*, purple trace, with the addition of 29.1 μM free bPEI polymer (grey traces). Spectral regions display slight peak changes for the corresponding labeled carbon residues. Abbreviations: GlcNAc, *N*-acetylglucosamine; ManNAc, *N*-acetylmannosamine; MurNAc, *N*-acetylmuramic acid; D-Ala, D-alanine; L-Ala, L-alanyl; *meso*-A_2pm, *meso*-diaminopimelyl; Glc, glucose; D-γ-Glu, D-γ-glutamyl; D-Lac, D-lactyl. WTA linker unit is composed of GlcNAc and ManNAc amino sugars.

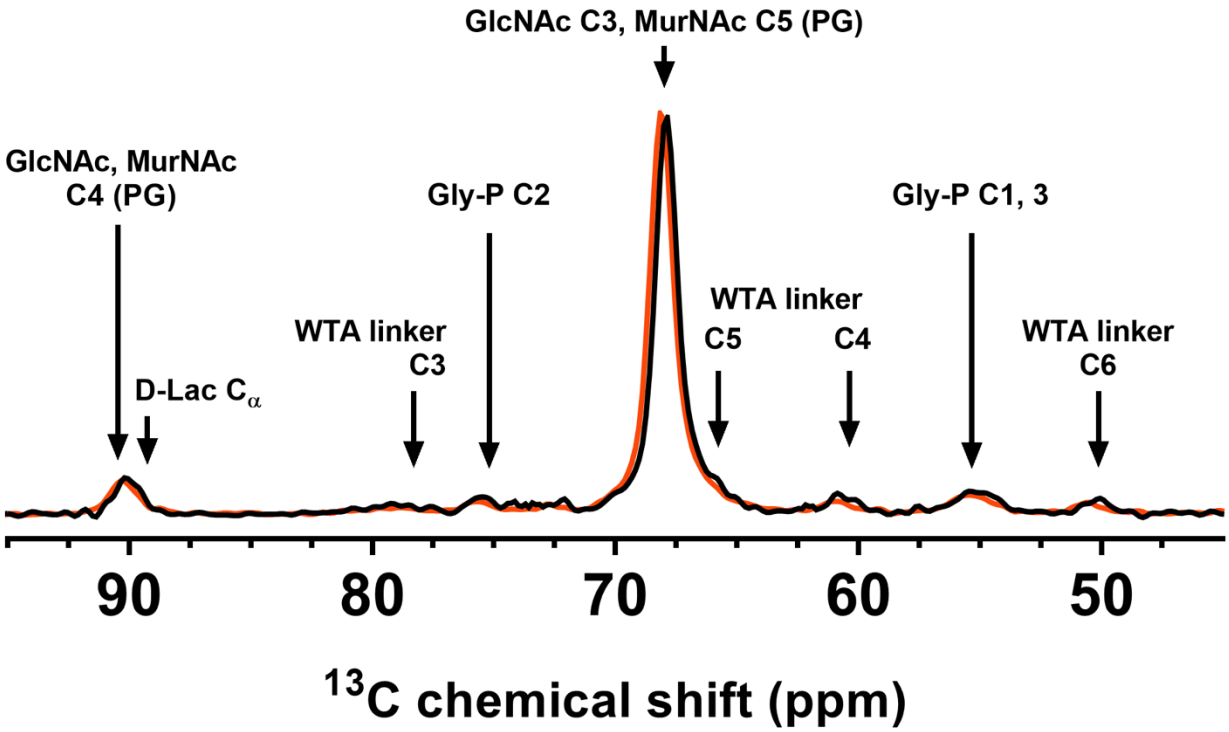


Fig. S11 Expanded region of solid-state ^{13}C-NMR spectrum of sacculi from Δ*dltA* (black trace) with the addition of 34 nM bPEI-AuNPs (orange trace). Spectral changes are minimal for the Δ*dltA* strain in the presence of both bPEI-AuNPs and free bPEI polymer (zoomed in region not shown). Abbreviations: GlcNAc, *N*-acetylglucosamine; ManNAc, *N*-acetylmannosamine; MurNAc, *N*-acetylmuramic acid; D-Lac, D-lactyl. WTA linker unit is composed of GlcNAc and ManNAc amino sugars.

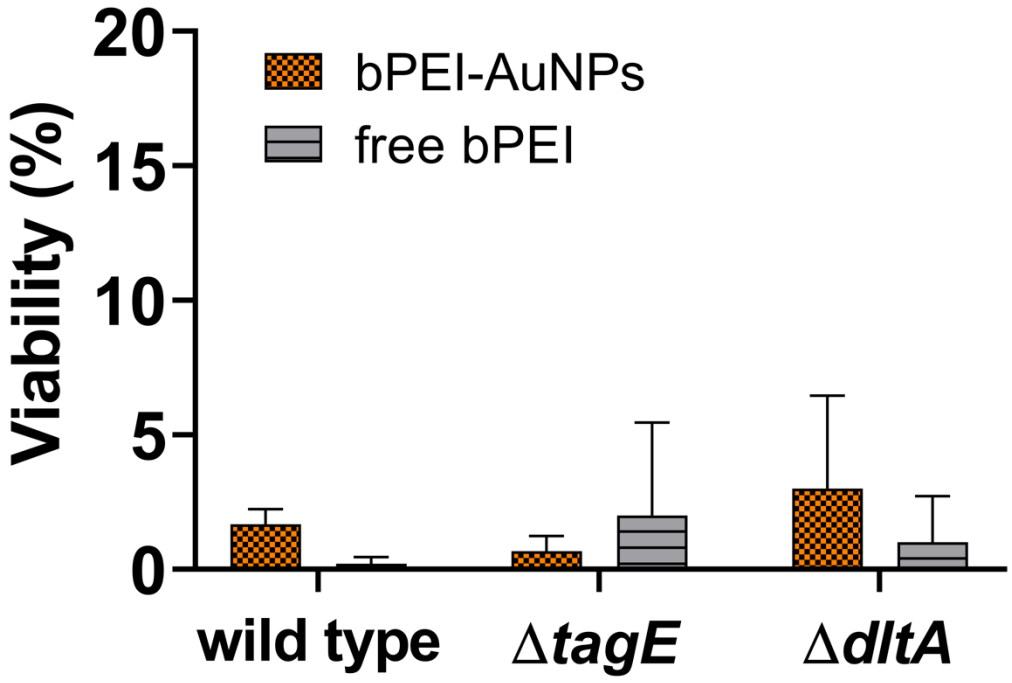


Fig. S12 Viability of *B. subtilis* mutants exposed to 0.93 nM bPEI-Au nanoparticles, and its equivalence of free bPEI polymers in solution (792 nM).

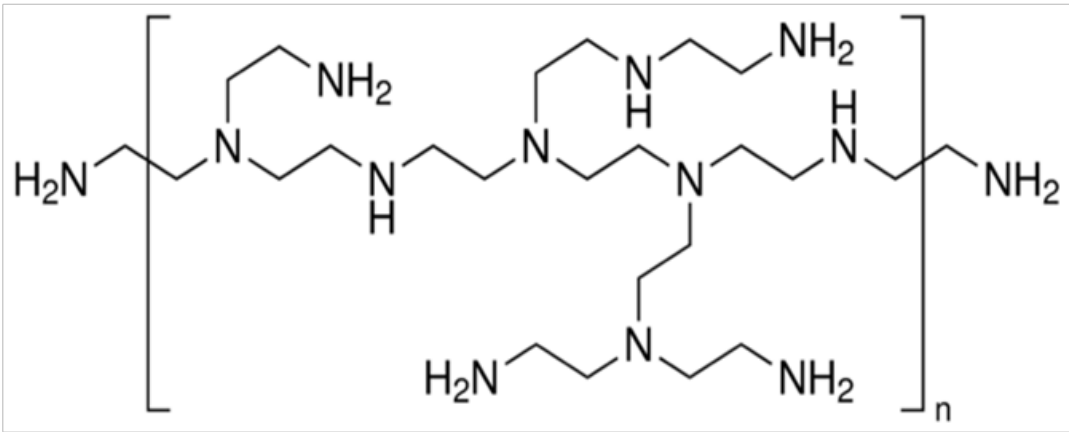


Fig. S13 Structure of branched polyethylenimine (bPEI).[13]

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