Supplemental Figures

for

Fluoresceination of FepA during Colicin B Killing: Effects of Temperature, Toxin and TonB

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[§] Contributed equally to this work

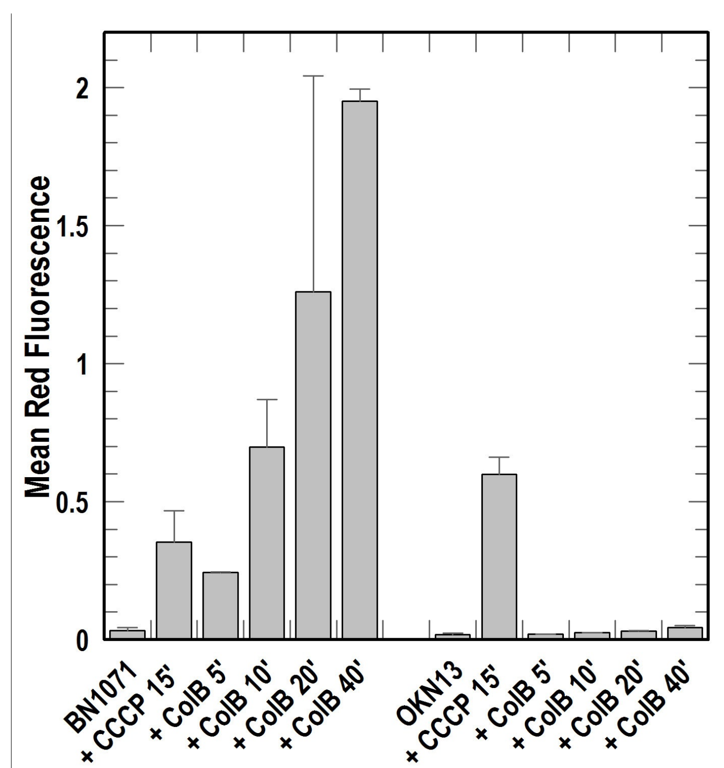

Figure S1. Kinetics of ColB-mediated lethality. *E. coli* strains BN1071 (*fepA*$^{+}$, *tonB*$^{+}$) and OKN13 (*fepA, tonB*) were grown overnight in LB broth, subcultured into MOPS minimal media and grown to mid-log phase, exposed to the depolarizing agents CCCP or ColB for the indicated times, stained with $DiOC_2(3)$, and cytofluorimetrically analyzed for red fluorescence. Depolarization of the bacterial cytoplasmic membrane results in an increase in red fluorescence of cell-associated $DiOC_2(3)$. CCCP depolarized both BN1071 and OKN13, whereas ColB only depolarized the former, *fepA*$^{+}$*tonB*$^{+}$ strain, within 40 minutes of addition of the toxin. The figure shows the mean fluorescence values from two experiments, and the associated standard errors of the means.

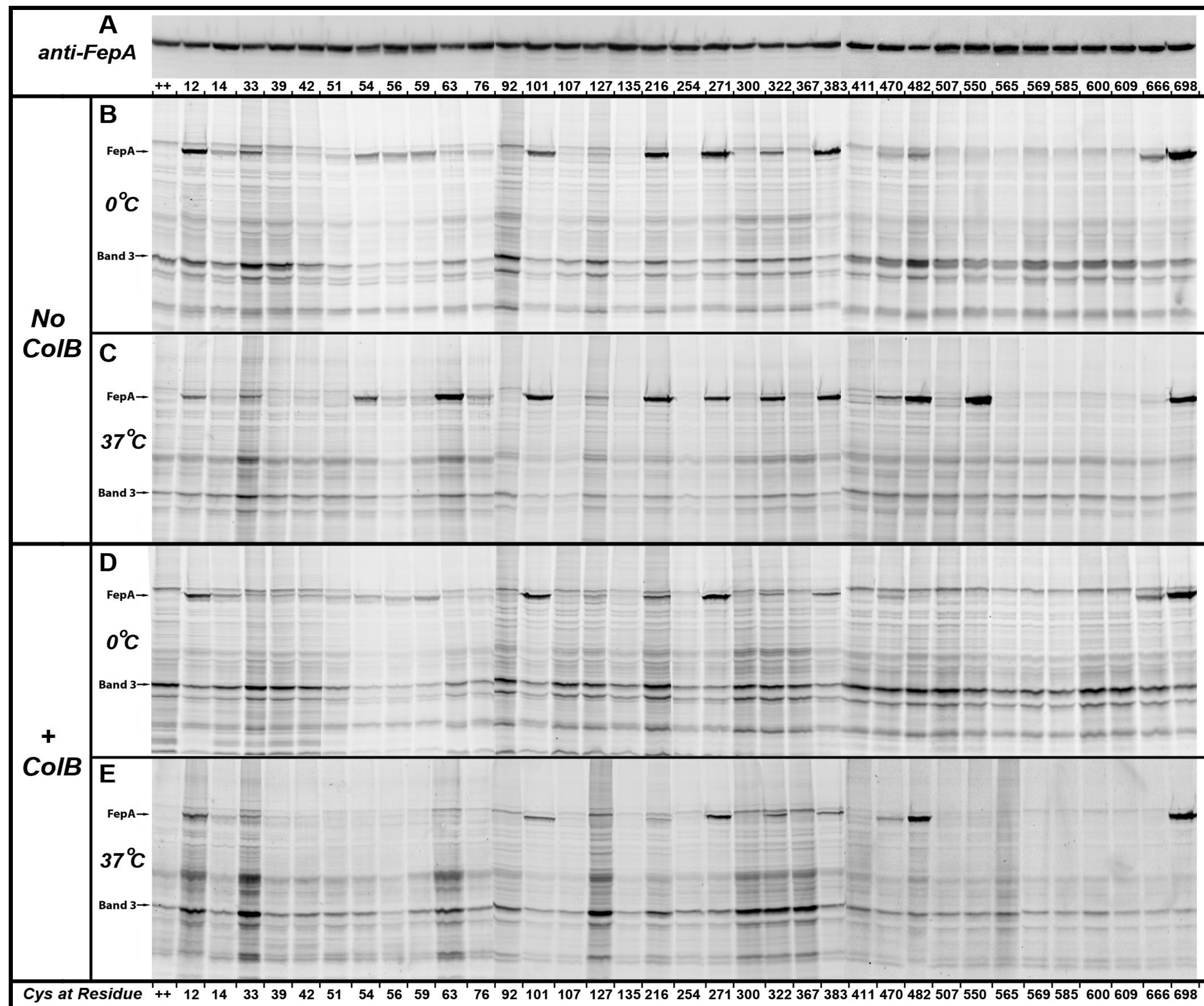
A
anti-FepA
++ 12 14 33 39 42 51 54 56 59 63 76 92 101 107 127 135 216 254 271 300 322 367 383 411 470 482 507 550 565 569 585 600 609 666 698
B
FepA
0°C
Band 3
No ColB
C
FepA
37°C
Band 3
D
FepA
0°C
Band 3
+ ColB
E
FepA
37°C
Band 3
Cys at Residue ++ 12 14 33 39 42 51 54 56 59 63 76 92 101 107 127 135 216 254 271 300 322 367 383 411 470 482 507 550 565 569 585 600 609 666 698

Figure S2. Representative images from scanned immunoblots and gels, which when quantified, produced Fig. 1. A. Expression. Synthesis of FepA was visualized by anti-FepA immunoblots. SDS-PAGE gels of fluoresceinated cell lysates were transferred to nitrocellulose, incubated with anti-FepA MAb 45 (Murphy et al., 1990), [^{125}I]-protein A (Newton et al., 1999), and scanned on a StormScan phosphorimager. In this panel and B-E, the composite image joins 3 separate immunoblots or SDS-PAGE gels (gel 1:residues 12-76; gel 2: 92-383; gel 3: 411-698) that were scanned on a StormScanner (Molecular Dynamics). **B. FM-labeling at 0 ºC.** After harvest bacteria were chilled to 0 ºC on ice, collected by centrifugation, resuspended in ice-cold PBS, incubated on ice for 30 min, and exposed to FM at 0 ºC for 15 min. **C. FM-labeling at 37 ºC**. Cells were manipulated as in panel B, but after resuspension in cold PBS they were incubated for 30 min at 37 ºC prior to exposure to FM at 37 ºC for 15 min. **D. FM-labeling at 0 ºC plus ColB.** Cells were manipulated as in panel B, but exposed to a 15-fold excess of ColB (relative to [FepA]) for 30 min at 0 ºC prior to exposure to FM at 0 ºC for 15 min. **E. FM-labeling at 37 ºC plus ColB.** Cells were manipulated as in panel B, but exposed to an 15-fold excess of ColB (relative to [FepA]) for 30 min at 37 ºC prior to exposure to FM at 37 ºC for 15 min. In different conditions, cells expressing single Cys mutants showed differential FM-labeling relative to the modification of Band 3 (Ma et al., 2006), which showed little variation in the samples (7.7 % ± 0.4% of total cellular fluorescence).

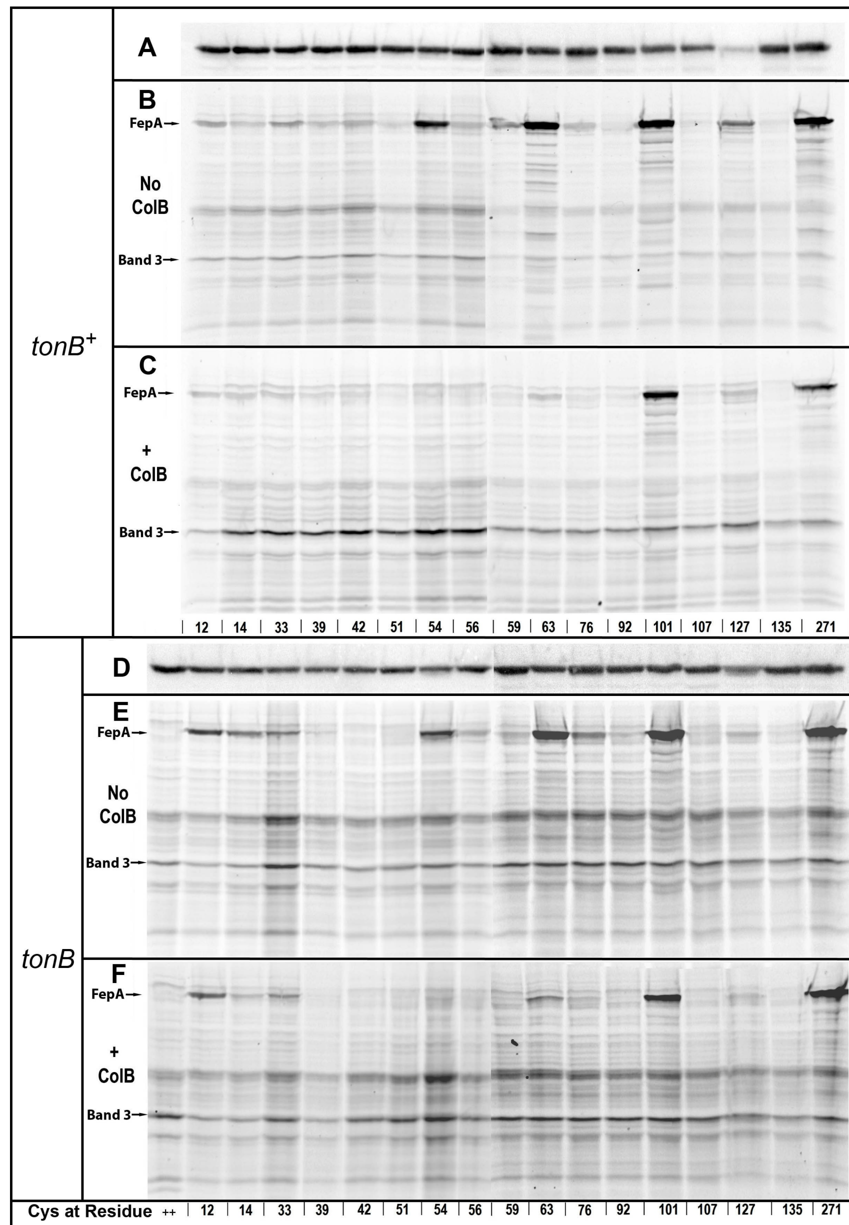

Figure S3. Representative images from immunoblots and gels, which when quantified, produced Fig. 2. A and D. Expression of FepA Cys mutants in OKN3 (*tonB*+; A*)* and OKN13 (*tonB*; D). Synthesis of FepA in was visualized as in Fig. S2. The images in these panels, and those in others in this figure, are composites from 2 separate immunoblots or gels (gel 1: residues 12-56; gel 2: 59-271); residue 271 is included as an internal positive control. The expression levels of wild-type FepA (++) and the Cys substitution mutant proteins (enumerated) were equivalent, but lower in OKN13 than in OKN3. **B and C. FM-labeling of Cys mutant proteins in OKN3 at 37 ºC, ± ColB.** Bacteria were prepared as in Fig. S2, but incubated at 37 ºC for 1 min in the absence (**B**) or presence (**C**) of ColB before FM was added for 15 min. **E and F. FM-labeling of Cys mutant proteins in OKN13 at 37 ºC, ± ColB.** Cells were manipulated as Fig. S2: after resuspension in cold PBS they were incubated for 30 min at 37 ºC in the absence (**E**) or presence (**F**) of ColB, prior to exposure to FM at 37 ºC for 15 min.

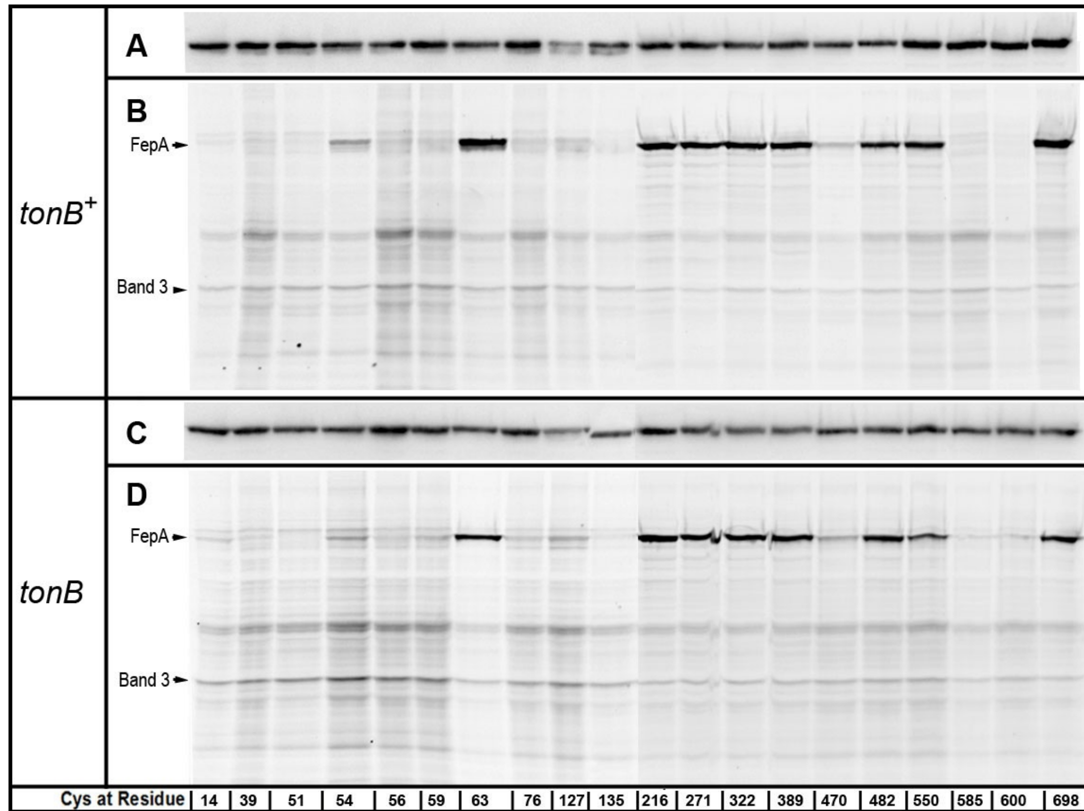

Figure S4. Representative images from scanned immunoblots and gels, which when quantified, produced Fig. 3. A and C. Expression of FepA Cys mutants in OKN3 (*tonB*+; A*)* and OKN13 (*tonB*; D). Synthesis of FepA in was visualized as in Fig. S2. The images in these panels, and those in other panels in this figure, are composites from 2 separate immunoblots or gels (gel 1: residues 14-135; gel 2: 216-298). The expression levels of the Cys substitution mutant proteins (enumerated) were equivalent, but lower in OKN13 than in OKN3. **B and D. Comparison of fluoresceination of Cys side chains in OKN3 (*tonB*$^+$; B) and OKN13 (*tonB*; D) bacteria at 37 ºC.** Cells were manipulated as Fig. S2: after resuspension in cold PBS they were subjected to FM-labeling at 37 ºC for 30 min.

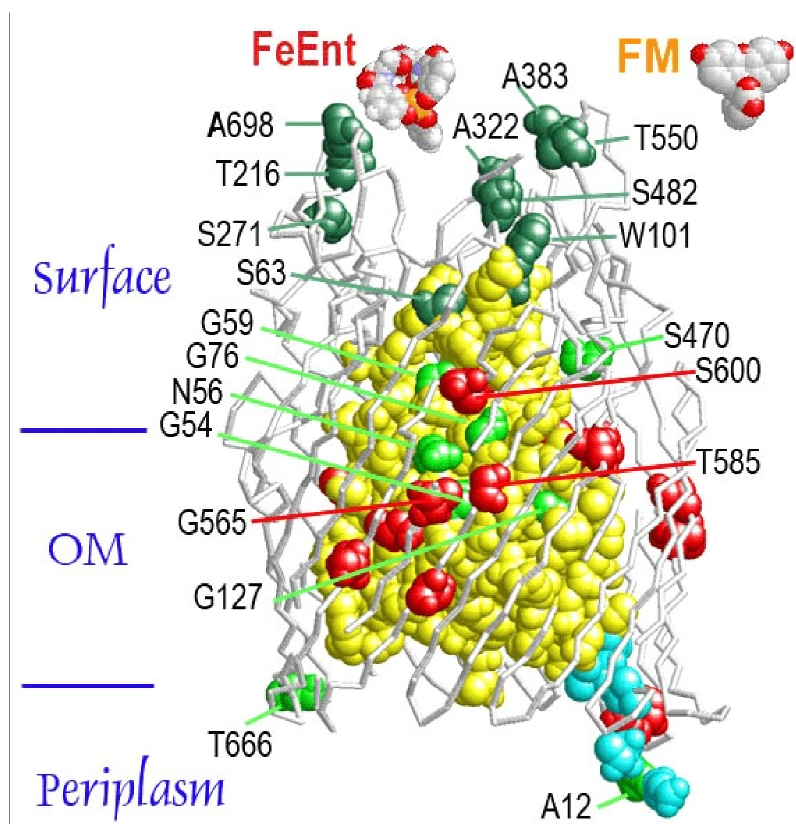

Figure S5. Relative accessibility of residues in FepA to modification by FM. A. The N-domain is yellow and the C-domain is white; the TonB-box (amino acids 11- 17) is cyan. **Left.** The positions of 27 Cys substitutions (among a total of 35, 8 are not discernible in this view) are shown in space-filling format on a backbone representation of FepA tertiary structure. The most reactive residues are colored dark green, and less intensely fluoresceinated residues are light green; for clarity, unreactive Cys substitutions (red) are not labeled.