Supporting Information

Jordan et al. 10.1073/pnas.1304243110

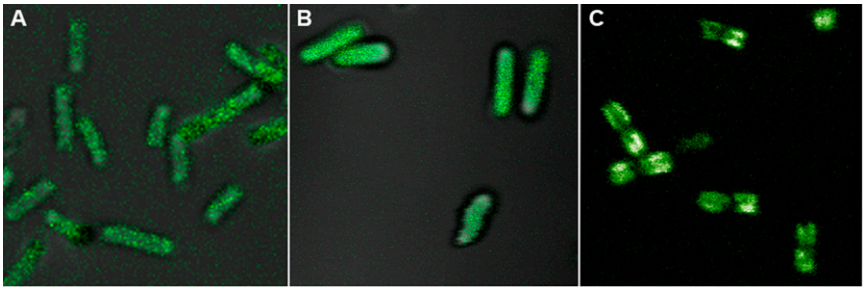


Fig. S1. Cellular distribution of GFP–protein fusions. (*A*) *Escherichia coli* strain T184/pT7GFP-LacY (kindly provided by Xiaoxu Jiang, Department of Physiology, UCLA School of Medicine, Los Angeles, CA) was grown in LB broth, induced with 0.1 mM isopropylthiogalactoside and observed by fluorescence microscopy. (*B* and *C*) *E. coli* strains BN1071/pTpG (1) and BN1071 *ΔexbBD*/pGT, respectively, were grown in LB broth, subcultured in MOPS medium, and observed by fluorescence microscopy. In *A* and *B*, GFP uniformly distributed throughout the cells, whereas in *C*, the fusion protein was restricted to the central regions of the cell, exactly as in wild-type bacteria.

1. Kaserer WA, et al. (2008) Insight from TonB hybrid proteins into the mechanism of iron transport through the outer membrane. *J Bacteriol* 190(11):4001–4016.

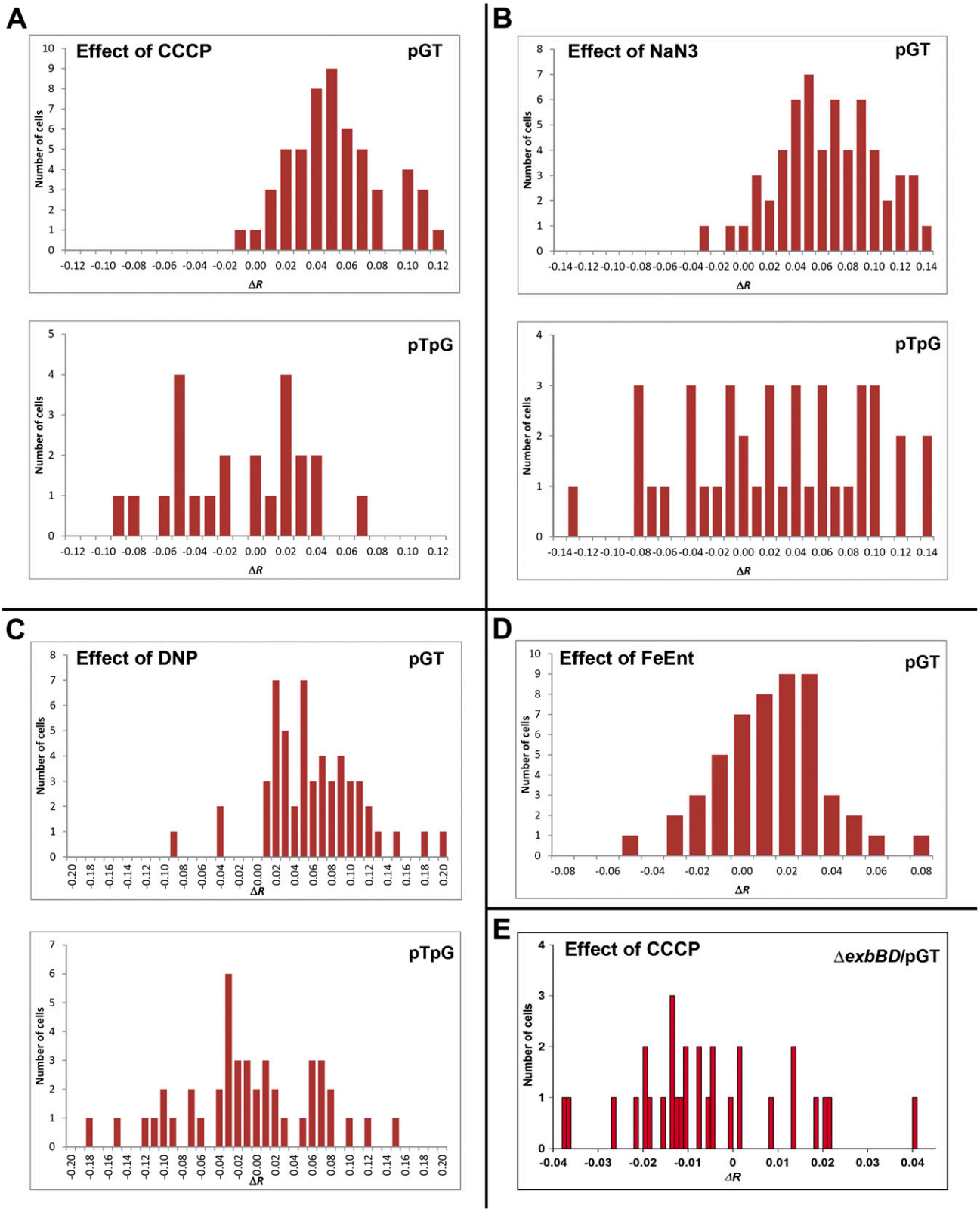


Fig. S2. Effect of metabolic inhibitors and ferric enterobactin (FeEnt) on the anisotropy of GFP and GFP-TonB. BN1071 harboring pTpG or pGT (*A–D*), and BN1071/*exbBD*/pGT (*E*) were grown and prepared for microscopy as described in Table 1. We measured anisotropy (*R*) in individual cells adhered to the coverslip, before and after the addition of the noted agents to the cuvette, and calculated Δ*R*. The histograms depict the data used to compile Table 1.

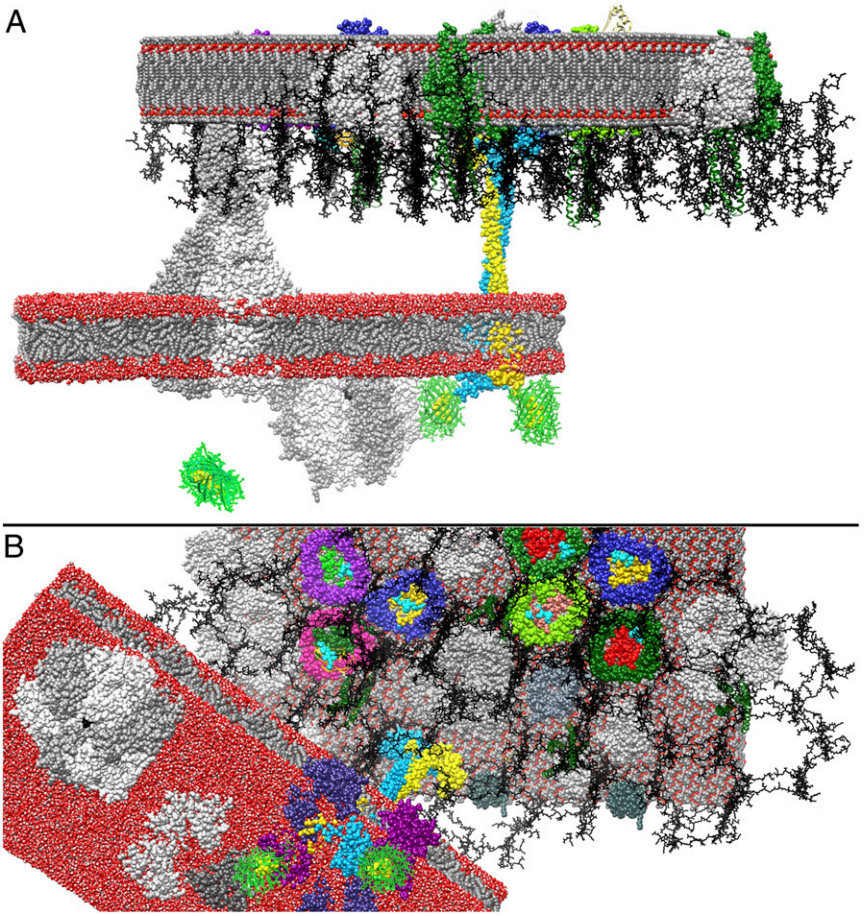
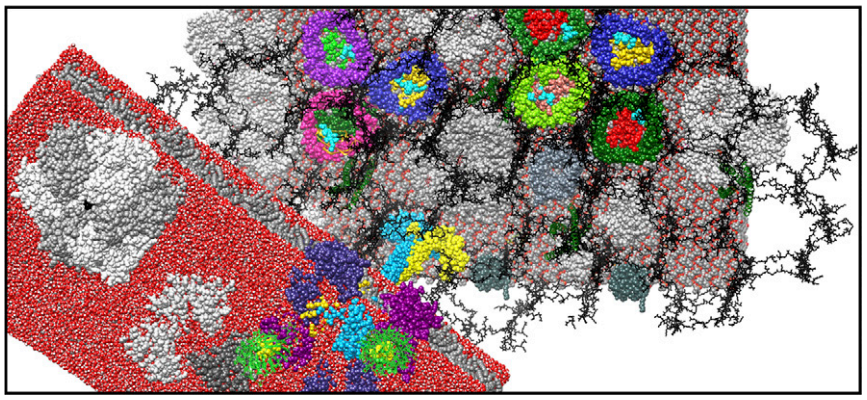


Fig. S3. Schematic representations of GFP-TonB in the Gram-negative bacterial cell envelope. (*A*) A cross-sectional view shows outer membrane (OM) proteins OmpF (gray) and FepA (dark green) spanning a frozen lipid bilayer, associated with peptidoglycan (PG; black wire frame) in the vertical scaffold model. The TolC–AcrAB complex (gray) across the periplasm defines the width of that space. We represented the TonB portion of GFP-TonB as a dimer (light blue and yellow), with its crystallographically defined C-terminus (1) contacting the inside of the OM, its central domain modeled as an extended coiled coil, and its N-terminus modeled as an integral domain in a fluid inner membrane (IM) bilayer. Green GFP β-barrels are fused to TonB N-termini and reside on the cytoplasmic face of the IM. ExbBD (magenta and purple, respectively) also are modeled as integral IM proteins, in contact with the TonB N-terminus. Note that crystallographic data do not yet exist for ExbBD or for the central region and N-terminus of TonB. The αβ trimer of the F1 subunit of the proton ATP-synthase is visible on the cytoplasmic surface of the IM. (*B*) A perspective from the cytoplasm shows the same components in the IM and a detailed view of the periplasmic surface of the OM. TonB-dependent proteins FepA (dark green and red), FecA (purple and green), FhuA (dark blue and yellow), Cir (light green and mauve), and BtuB (pink and dark green), and other proteins OmpF, LamB, OmpA, OmpG, OmpT (all in shades of gray), lipoprotein (dark green helices) organized in the hexagonal cells of the PG matrix. In the motor model, proton motive force rotates the TonB N-terminus within an oligomeric ExbBD stator in the IM bilayer, moving the attached GFP subunits in the cytoplasm and the dimeric TonB C-terminus in contact with the OM surface (Movie S1). Crystallographic coordinates of the component molecules [from the Protein Data Bank (PDB)] were rendered by Chimera [University of California, San Francisco (UCSF)].

1. Chang C, Mooser A, Plückthun A, Wlodawer A (2001) Crystal structure of the dimeric C-terminal domain of TonB reveals a novel fold. *J Biol Chem* 276(29):27535–27540.



Movie S1. Movie depiction of proposed TonB motion in Gram-negative bacteria. The molecules comprising this schematic representation are described in Fig. S3; their crystallographic coordinates (from the PDB database) were rendered by Chimera (UCSF). The results indicate that ExbBD (magenta and purple, respectively) in the IM bilayer create a functional link to the electrochemical gradient that ultimately energizes the motion of the TonB dimer (light blue and yellow). The movements of TonB transfer energy across the periplasmic space and facilitate metal transport by causing conformational changes in OM transporters as its C-terminus interacts with their TonB-box regions. The movie does not depict an additional aspect of the model: the TonB–ExbBD complex may undergo rotation-driven lateral motion in the fluid IM bilayer that moves the TonB C-terminus through the PG matrix, allowing surveillance of the inner surface of the OM for ligand-bound iron transporters.

Movie S1