## SI Appendix

## Raina, Aoyama et al.

**Bacterial Strains and Plasmids.** Bacterial strains, plasmids, and oligonucleotides used in this study are listed in Dataset S1. *E. coli* strains are derivatives of wild-type MG1655 (F- lambda- *ilvG- rfb*-50 *rph*-1). Tagged strains were generated by λ Red–mediated recombineering (1) using NM400 and the oligonucleotides listed in Appendix Table S3. pJL148 (2) was used as the template to amplify the SPA tag. The chromosomal $P_{BAD}$-5′UTR$_{azuC}$-*lacZ* and $P_{BAD}$-5′UTR$_{azuC}$-*lacZ* III fusions (carrying the first 87 nt of the AzuCR RNA fused to the seventh codon of the *lacZ* coding sequence) were created by carrying out PCR using primers listed in Appendix Table S3 to amplify the desired region of *azuC* followed by integration of the product into the chromosome of PM1205 (3). Alleles marked by antibiotic markers were moved between strains by P1 transduction. When necessary, kanamycin resistance cassettes were excised from the chromosome by FLP-mediated recombination using the FLP recombinase encoded on pCP20 (4). All plasmids are derivatives of pAZ3 (5), pKK177-3 (6), pRI (7), pBRpLac (8) or pXG10-SF (9). All chromosomal mutations and fusions and plasmid inserts were confirmed by sequencing.

**Bacterial Growth Conditions.** Cells were grown in Luria-Bertani broth (LB) or M63 minimal media supplemented with 0.001% vitamin B1 and glucose, glycerol or galactose (0.2%, 0.4% or 0.2%, respectively). Unless stated otherwise, M63 medium was unbuffered at pH 7.0. For some experiments, M63 medium was buffered to pH 5.5 with 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES). Cells were grown to the indicated $OD_{600}$ after dilution of

an overnight culture grown in LB, except for cells grown in M63 glycerol, pH 5.5 samples, where overnight cultures were grown in M63 glycerol, pH 5.5. Generally, overnight cultures were diluted 1:100. However, to be able to collect cells at the desired $OD_{600}$ in a reasonable time frame, some overnight cultures were diluted more than 1:100. Where indicated, media contained antibiotics with the following concentrations: ampicillin (100 μg/ml), chloramphenicol (25 μg/ml) and kanamycin (30 μg/ml).

**Immunoblot Analysis.** The cell pellet from 1 ml of cells grown in the indicated medium was resuspended in 1X PBS (KD Medical), 7 μl of 2X Laemmli buffer (BioRad) and 2 μl of β-mercaptoethanol (βME), and 10 μl were loaded on a Mini-PROTEAN TGX 5%–20% Tris-glycine gel (Bio-Rad) and run in 1X Tris-glycine-SDS (KD Medical) buffer. The proteins were electro-transferred to nitrocellulose membranes (Invitrogen) for 1 h at 100 V. Membranes were blocked in 1X PBS 0.1% of Tween 20 (PBS-T) with either 5% milk (Bio-Rad, for α-FLAG antiserum) or 5% BSA (for all other antibodies) for 1 h and and probed with a 1:3,000 dilution of α-FLAG-HRP antiserum (Sigma); a 1:1,000 dilution of α-AzuC antiserum (New England Peptide); 1:1,000 dilution of α-His-HRP antiserum (Qiagen) or 1:1,000 dilution of α-OmpA antiserum (Antibody Research Corporation) in PBS-T with 5% milk or 5% BSA as above for 1 h or longer. After the incubation with the α-AzuC or α-OmpA antiserum, membranes were incubated with a 1:2,000 dilution of HRP-labelled anti-rabbit antibody (Life Technologies). All blots were washed 4X with PBS-T and then developed with an Amersham ECL Western Blotting Detection Kit (GE Healthcare).

**RNA isolation.** Cells corresponding to the equivalent of 10 $OD_{600}$ were collected by centrifugation, and snap frozen in liquid nitrogen. RNA was extracted according to the standard TRIzol (Thermo Fisher Scientific) protocol. Briefly, 1 ml of room temperature TRIzol was add to cell pellets, resuspended thoroughly to homogenization, and incubated for 5 min at room temperature. After the addition of 200 µl of chloroform and thorough mixing by inversion, samples were incubated for 10 min at room temperature. After samples were centrifuged for 10 min at 4˚C on maximal speed, the upper phase (~0.6 ml) was transferred into a new tube and 500 µl of isopropanol was added. Samples again were mixed thoroughly by inversion, incubated for 10 min at room temperature and centrifuged at maximal speed for 15 min at 4˚C. RNA pellets were washed twice with 75% ethanol and then dried at room temperature. RNA was resuspended in 20-50 µl of DEPC water and quantified using a NanoDrop (Thermo Fisher Scientific).

**Northern Analysis.** Total RNA (5-10 µg per lane) was separated on denaturing 8% polyacrylamide gels containing 6 M urea (1:4 mix of Ureagel Complete to Ureagel-8 (National Diagnostics) with 0.08% ammonium persulfate in 1X TBE buffer at 300 V for 90 min. The RNA was transferred to a Zeta-Probe GT membrane (Bio-Rad) at 20 V for 16 h in 0.5X TBE, UV-crosslinked, and probed with $^{32}$P-labeled oligonucleotides (Listed in Table 1) in ULTRAhyb-Oligo buffer (Ambion Inc.) at 45˚C. Membranes were rinsed twice with 2X SSC-0.1% SDS at room temperature, once with 0.2X SSC-0.1% SDS at room temperature, washed for 25 min with 0.2X SSC-0.1% SDS at 45˚C, followed by a final rinse with 0.2X SSC-0.1% SDS at room temperature before autoradiography was performed with HyBlot CL film (Denville Scientific Inc.).

**Sub-cellular Fractionation.** Cells with chromosomally-encoded AzuC-SPA were grown in the indicated medium at 37˚C to an $OD_{600}$ ~0.3, centrifuged at 20,000 × g for 10 min at 4˚C, resuspended in fractionation buffer (1/20 vol of 20% sucrose, 50 mM Tris-HCl pH 8.0) with 1 mM EDTA and 0.1 mg/ml lysozyme, and then incubated 1 h at 25˚C with gentle shaking. After the cells were centrifuged at 20,000 × g for 15 min at 4˚C, the top periplasmic fraction was removed. The pellet fraction was resuspended in water to lyse the spheroplasts. The resulting crude lysate was passed through a 30-gauge syringe needle 6X to homogenize the sample and reduce viscosity. The lysate was then clarified by centrifugation at 20,000 × g for 5 min at 4˚C. This was repeated 3X. A 500 µl of the clarified lysate was layered on top of a 500 µl-sucrose cushion (5 mM EDTA and 1.4 M sucrose. Samples were centrifuged at 130,000 × g for 2 h at 4˚C in a TLA100.3 rotor (Beckman Optima TLX tabletop centrifuge). Following centrifugation, 425 µl was carefully removed from the top layer (soluble fraction). Then, the interface and remaining liquid were removed (inner membrane fraction). The pelleted material was resuspended in 500 µl of fractionation buffer (pellet fraction). SDS was added to all fractions (final concentration 1%) and the samples were incubated overnight at room temperature. Equal volumes of fractions were assayed by immunoblotting with α-FLAG-HRP and α-OmpA antibody.

Cells expressing AzuC from a plasmid were grown as above, collected by centrifugation at 4K rpm for 10 min at 4˚C, resuspended as above but incubated 10 min on ice. After the lysate was incubated as above the periplasmic fraction removed, the pellet was resuspended in 1 ml of 20% sucrose, 50 mM Tris-HCl pH 8.0 and sonicated with a Sonic Dismembrator Model 100 (Fisher Scientific) 3X for 5 sec at power setting 4. Samples were centrifuged 3X at 12,000 × g for 5 min at 4˚C to remove unlysed cells. The supernatant was then centrifuged at 56K rpm for 1

h at 4˚C in a Beckman TLA100.3 rotor. The supernatant containing the cytoplasmic fraction was removed and the pellet containing the membrane fraction was resuspended in 1 ml of 20% sucrose, 10mM Tris-HCl pH 8.0 by sonication. Equal volumes of fractions were assayed by immunoblotting with polyclonal α-AzuC antibody.

**Microscopy.** Cells grown as indicated were harvested, washed 1X in phosphate buffered saline (PBS) (KD Medical), resuspended in PBS, and placed on lysine-coated glass bottom dish (Mattek Corporation). Cells were fixed by applying a 1% agarose pad on top of the sample with gentle pressure. Cells were viewed with a DeltaVision Core microscope system (Applied Precision) equipped with an environmental control chamber. Bright field and fluorescence images were captured with a Photometrics CoolSnap HQ2 camera. Seventeen planes were acquired every 0.2 μm at 22˚C, and the data were deconvolved using SoftWorx software (GE Healthcare). For the quantitation of GlpD-YFP localization, cells of the indicated strains, as well as MG1655 control cells, were grown in M63 glycerol at pH 5.5 for 16 h. Prior to imaging, the MG1655 control cells were mixed at a 1:1 ratio with either of the two GlpD-YFP strains. To compare the membrane-associated YFP intensity, the relative intensity was calculated by dividing the average intensity for 10 YFP-labeled cells by that for 10 unlabeled MG1655 cells (in each case determined for a defined volume crossing the membrane) for four independent images.

**Purification of Chromosomally-Encoded AzuC-SPA.** Cells expressing AzuC-SPA (GSO351) cells grown in LB at 37˚C overnight culture were diluted 1:100 into 1 l of M63 glucose minimal media and incubated at 37˚C. At $OD_{600}$ ~1.0, cells were collected by centrifugation (4,650 × g,

20 min). The pellet was resuspended in 20 ml of TNG buffer [10 mM Tris-HCl pH 7.5, 100 mM NaCl, 10% glycerol] supplemented with Protease Inhibitor Cocktail (Roche). The cells were lysed using a microfluidizer processor (Microfluidics) at 20,000 psi, and the insoluble cellular debris was removed by centrifugation (20,000 × g, 30 min). The cleared lysate was incubated with 50 mM dodecyl β-D-maltoside (DDM) at 4˚C for 2 h. Next, the DDM-supplemented lysate was incubated with 500 µl of calmodulin-sepharose beads (Amersham Biosciences) overnight at 4˚C. The lysate and beads were applied to a Bio-Spin disposable chromatography column (Bio-Rad Laboratories) and allowed to drain by gravity. The calmodulin column was washed with 15 ml of TNG buffer with 2 mM DDM, 5 mM β-ME, and 2 mM CaCl. Finally, proteins were eluted from the calmodulin column in 1 ml TNG buffer supplemented with 4 mM EDTA, 5 mM β-ME, and 2.5% SDS. To analyze the protein samples, 7.5 µl of 2X Laemmli buffer was added to 21 µl of each sample. The samples were heated at 95˚C for 5 min, and aliquots were subjected to SDS/PAGE in a 10–20% Tris-glycine gel (Invitrogen) at 12 V/cm. Proteins were visualized with Coomassie Blue Stain. Bands of interest were excised from the gel and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). An identical purification was carried out for cells with chromosomal *acrZ-SPA* (GSO350) grown in 1 l of LB to $OD_{600}$ ~0.6.

**Reciprocal Purification.** For cell mixing experiments, MG1655 cells or cells expressing AzuC-SPA (GSO351), GlpD-HA-$His_6$ (GSO1011) or the control MgtA-HA (GSO785) from the chromosome were grown in LB at 37˚C for 16 h, and then diluted 1:100 into 1 l of M63 glucose minimal medium, M63 glycerol minimal medium or N medium with 500 µM $MgSO_4$, respectively and incubated at 37˚C. The WT strain and strains expressing GlpD-HA-$His_6$ and AzuC-SPA were grown to $OD_{600}$ ~1.0. The strains expressing MgtA-HA were grown to $OD_{600}$

~0.4–0.6, collected, washed 2X in N medium without added $MgSO_4$, resuspended in N medium without added $MgSO_4$ and grown for another 2.5 h to induce MgtA-HA expression. For all cultures, cells were collected by centrifugation (4,650 × g, 20 min) and resuspended in 15 ml of TNG buffer supplemented with Protease Inhibitor Cocktail (Roche). Cells from the SPA-tagged protein cultures were mixed with the control WT or HA-tagged protein cultures at a 1:1 ratio. To ensure thorough mixing, cells were shaken gently at 4˚C for 15 min. For experiments, where AzuC-SPA and GlpD-HA-$His_6$ were expressed in the same cell, overnight cultures grown in M63 glycerol pH 5.5 were diluted 1:100 into 1 l of M63 glycerol pH 5.5 and grown to $OD_{600}$ ~1.5. For all purifications, cells were homogenized as for the SPA-tagged protein purification and incubated with 50 mM DDM in 4˚C for 2 h. The insoluble cellular debris was removed by centrifugation (20,000 × g, 20 min). For co-purification with the HA-tagged proteins, the supernatant was applied to 100 µL of Pierce α-HA magnetic beads (Thermo Scientific) in a 50 ml tube and incubated overnight at 4˚C. Beads were collected with a MagneSphere technology magnetic separation stand (Promega) and resuspended in 1 ml of TNG buffer. The beads were washed with 1 ml of TNG buffer (10X). The beads were then resuspended in 1X PBS (50 µl) and 2X Laemmli buffer (50 µl) and heated at 95˚C for 5 min. For co-purification with FLAG-tagged proteins, the supernatant was applied to 100 µl of Pierce α-FLAG affinity resin (Thermo Scientific) in a 15 ml tube and incubated overnight at 4˚C. Resin was collected with light centrifugation (1,000 x g, 1 min) and washed with 1 ml of TNG buffer (3X). The resin was then eluted in 200 mM glycine (pH 7). Samples (15 µl) were analyzed on immunoblots using α-His or M2 α-FLAG antibodies.

**Dehydrogenase Activity Assay.** Cells were grown in M63 glucose minimal medium to $OD_{600}$ ~0.5. Cells were pelleted and washed with M63 glycerol medium, pH 7.0 or pH 5.5. Cells were then resuspended in same volume of the same medium and grown at 37˚C for 3 h. Cells (500 µl) were pelleted and resuspended in 500 µl of lysis buffer (25 mM Tris-HCl pH 7.5, 10 mM NaCl and 0.4% Triton X100). Cells were lysed by adding 0.6 g of glass beads and vortexing 30 s followed by 30 s incubation on ice, repeated 5X. The cells were then centrifuged at 20,000 × g for 2 min at 4˚C, and the lysate was used to measure the dehydrogenase activity. A method monitoring 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-2*H*-tetrazolium bromide (MTT) reduction to quantitate the dehydrogenase activity of GlpD (10) was modified as follows. Each 225 µl microcuvette contained the following: 25 mM Tris-HCl pH 7.5, 100 mM NaCl, 1 mM MTT (Sigma Aldrich), 3 mM phenazine methosulfate (PMS, Sigma Aldrich) and 100 µl of lysate. This was used as the blank, and the reaction was initiated by the addition of 3.7 mM *sn*-glycerol-3-phosphate (Sigma Aldrich). The reduction of MTT at 570 nm was continuously monitored on a BMG LABTECH plate reader for 118 min at room temperature.

**β-Galactosidase Assays.** Cultures were grown in LB to $OD_{600}$ ~1.0 with arabinose (0.2%). 100 µl of cells were added to 700 µl of Z buffer (60 mM $Na_2HPO_4$, 40 mM $NaH_2PO_4$, 10 mM KCl, 1 mM $MgSO_4$, 50 mM βME). After the addition of 15 µl of freshly prepared 0.1% SDS and 30 µl of chloroform, each sample was vortexed for 30 s and then incubated at room temperature for 15 min to lyse the cells. The assay was initiated by adding 100 µl of ONPG (4 mg/ml). The samples were incubated at room temperature until the reaction was terminated by the addition of 500 µl of 1M $Na_2CO_3$. $A_{420}$ and $A_{550}$ values determined with a spectrophotometer were used to calculate Miller units.

**Growth Curves.** Colonies of Δ*azuCR::kan* (GSO193) transformed with pRI, pRI-AzuCR, pRI-AzuCR$_{L3STOP}$, pKK, pKK-AzuC or pKK-AzuC$_{L3STOP}$ grown on LB plates were inoculated into glucose (pH 7.0), glycerol (pH 7.0 and 5.5), and galactose (pH 7.0) and allowed to grow overnight at 37˚C, at which point all cultures were in stationary phase. Cultures were diluted to $OD_{600}$ ~0.05 (time 0) in 25 ml of the same media and grown at 37˚C. $OD_{600}$ was measured at 16 h or growth was followed for 29 h.

**GFP Reporter Assay.** The GFP reporter assay was principally done as described previously (9, 11). WT or Δ*azuCR::kan* (GSO193) cells were transformed with a *cadA-gfp*, *cadA-gfp-M1*, *galE-gfp* or *galE-gfp-M2* reporter plasmid and a pRI-AzuCR, pRI-AzuCR$_{L3STOP}$, pRI-AzuCR$_{L3STOP}$-M1 or AzuCR$_{L3STOP}$-M2 over-expressing plasmid or pRI as a control. Single colonies were grown overnight at 37˚C in LB supplemented with ampicillin and chloramphenicol. The cultures were diluted to $OD_{600}$ ~0.05 in fresh medium and grown at 37˚C for 3 h in a 96 deep-well plate. An aliquot (1 ml) of each culture was centrifuged and the pellet was resuspended in 220 µl of 1X PBS. Fluorescence was measured using the CytoFLEX Flow Cytometer (Beckman Coulter). Three biological repeats were analyzed for every sample.

**Hfq and ProQ Co-Immunoprecipitation Assays.** Cell extracts were prepared from MG1655 cells grown in M63 glucose medium to $OD_{600}$ ~0.5. Cells corresponding to the equivalent of 20 $OD_{600}$ were collected, and cell lysates were prepared by vortexing with 212-300 µm glass beads (Sigma-Aldrich) in a final volume of 1 ml lysis buffer (20 mM Tris-HCl pH 8.0, 150 mM KCl, 1 mM $MgCl_2$, 1 mM DTT). Immunoprecipitations were carried out using 100 µl of Hfq antiserum

(12) or 100 µl of ProQ antiserum (13), 120 mg of protein-A-sepharose (Amersham Biosciences, Piscataway, NJ) and 950 µl of cell extract per immunoprecipitation reaction. Immunoprecipitated RNA was isolated from immunoprecipitated pellets by extraction with phenol:chloroform:isoamyl alcohol (25:24:1, pH 8), followed by ethanol precipitation. Total RNA was isolated from 50 µl of cell lysate by Trizol (Thermo Fisher Scientific) extraction followed by chloroform extraction and isopropanol precipitation. Total and co-IP RNA samples were resuspended in 20 µl of DEPC $H_2O$ and 2 µg of total RNA or 200 ng of IP RNA was subjected to Northern analysis as described below.


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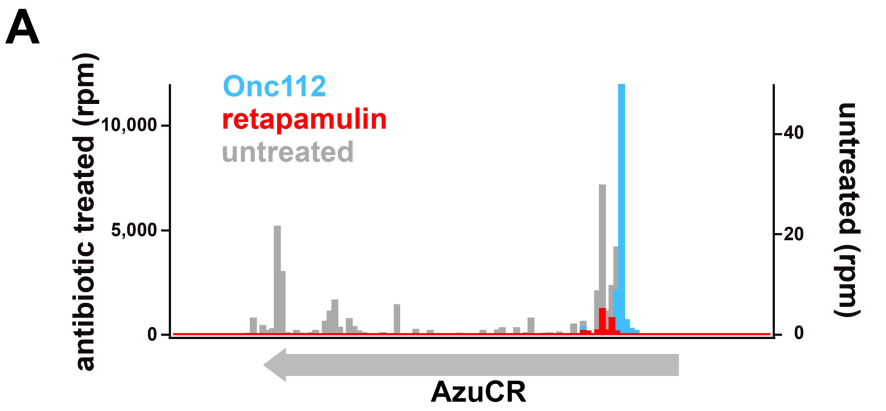


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Enterobacter_cloacae            --MKLRKILKNMWANYCNTFKDVPPGAMF-    27
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Mangrovibacter_plantisponsor    --MKLRRILKSMFENYCKTFKDVPPGAMF-    27
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**Fig. S1.** Ribosome binding to and conservation of *azuC*. (*A*)The *azuC* open reading frame lies within a region that was previously reported to encode the IsrB sRNA (14), here denoted AzuCR (denoted by the arrow). Translation is detected by ribosome density on the *azuC* gene for an untreated control (gray) (15), and cells treated with the translation inhibitors Onc112 (blue) (15) or retapamulin (red) (16). (*B*) The AzuC amino acid sequences from *E. coli* K12 and other bacterial species aligned with ClustalW (17). "*" indicates the residues are identical in all sequences and ":" and "." respectively indicate that conserved and semi-conserved substitutions as defined by ClustalW.

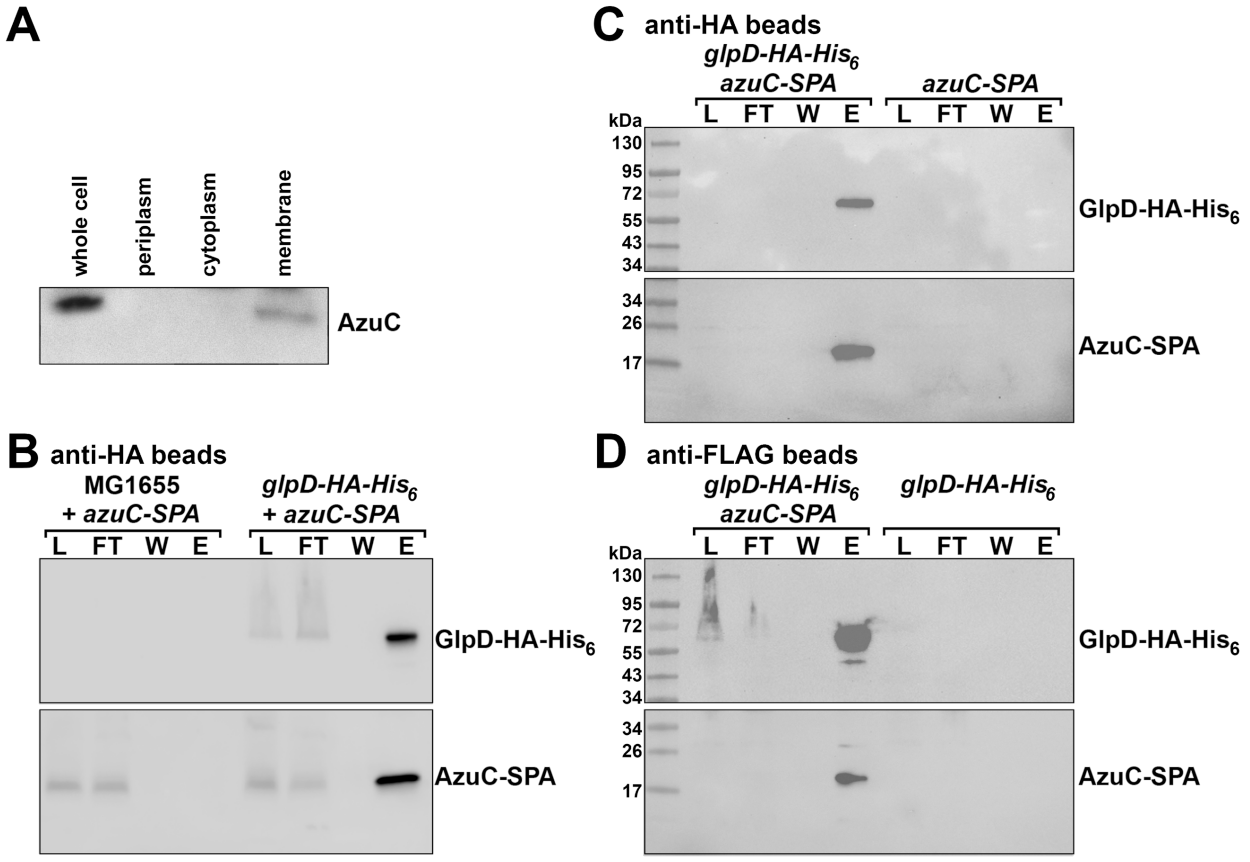


**Fig. S2.** Fractionation showing subcellular localization of untagged AzuC and AzuC-SPA co-purification with GlpD-HA-$His_6$ compared to untagged control strain. (*A*) AzuC was overexpressed at low levels from the arabinose-inducible $P_{BAD}$ promoter on the multicopy pAZ3 plasmid derivative of pBAD18 (5). After induction with arabinose, cell extracts were fractionated into periplasmic, cytoplasmic, and membrane fractions. The fractions were then examined on immunoblots using polyclonal α-AzuC primary antibody followed by α-rabbit secondary antibody. The polyclonal α-AzuC antiserum is not very avid and has a high background so AzuC expressed from the chromosome could not be detected. (*B*) AzuC-SPA co-purifies with GlpD-HA-$His_6$ from mixed cells. AzuC-SPA cells grown in M63 glucose and MG1655 cells or GlpD-HA-$His_6$ cells grown in M63 glycerol, to $OD_{600}$ ~1.0 were mixed in a 1:1

ratio. The mixed cells were homogenized, cell lysates (L) were applied to anti-HA beads and the flow-through (FT) fractions were collected. (*C*) AzuC-SPA co-purifies with GlpD-HA-$His_6$ when both are expressed from the chromosome in the same cell. Cells expressing both AzuC-SPA and GlpD-HA-$His_6$ (GSO1118) or just AzuC-SPA (GSO351) were grown in M63 glycerol media (pH 5.5) to $OD_{600}$ ~1.5. Cell lysates (L) were applied to α-HA beads and the flow-through (FT) samples were collected. (*D*) GlpD-HA-$His_6$ co-purifies with AzuC-SPA when both are expressed from the chromosome in the same cell. Cells expressing both AzuC-SPA and GlpD-HA-$His_6$ (GSO1118) or just GlpD-HA-$His_6$ (GSO1011) were grown in M63 glycerol media (pH 5.5) to $OD_{600}$ ~1.5. Cell lysates (L) were applied to α-FLAG beads and the flow-through (FT) samples were collected. For (*B*), (*C*) and (*D*), the respective beads were washed (W), after which the bound proteins were eluted (E). All samples were examined on immunoblots using either α-His antibodies to detect GlpD-HA-$His_6$ (top panel) or α-FLAG antibodies to detect AzuC-SPA (bottom panel).

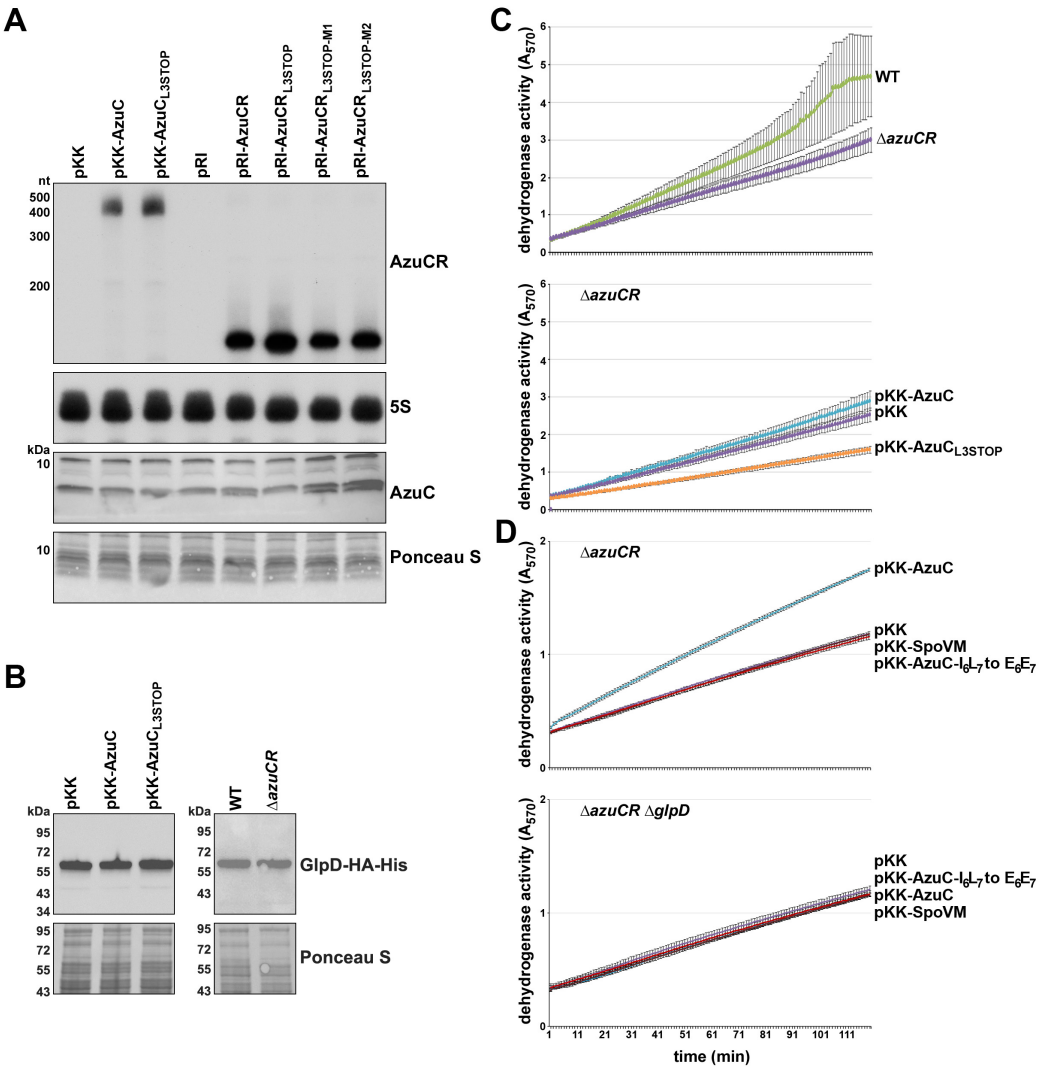


**Fig. S3.** Effect of AzuC overexpression on GlpD levels at pH 5.5 and on GlpD activity at pH 7.0. (*A*) Levels of AzuCR mRNA and AzuC protein expressed from pKK and pRI overexpression plasmids. Δ*azuCR* cells transformed with pKK, pKK-AzuC, pKK-AzuC$_{L3STOP}$, pRI, pRI-AzuCR, pRI-AzuCR$_{L3STOP}$, pRI-AzuCR$_{L3STOP-M1}$, or pRI-AzuCR$_{L3STOP-M2}$ were grown in LB medium to OD$_{600}$ ~ 0.5. For the top panels, RNA was extracted and analyzed with northern analysis using an oligonucleotide specific for AzuCR or the 5S RNA. We note that this AzuC RNA is longer than expected and longer than the AzuCR RNA. We suggest this is because the strong AzuCR

terminator has been removed in the pKK-AzuC construct, so the transcript terminates at the *rrnB* terminator in the plasmid and thus contains some additional plasmid sequence. For the bottom panels, total protein was examined by an immunoblot assay using α-AzuC primary antibodies and goat α-rabbit secondary antibodies to detect AzuC protein. The polyclonal α-AzuC antiserum is not very avid and has a high background so overexpressed AzuC is barely detected below a background band. The membrane was stained with Ponceau S stain as a loading control. (*B*) Levels of GlpD-HA-$His_6$ in cells with increased or decreased AzuC expression. Δ*azuCR* cells expressing GlpD-HA-$His_6$ (GSO1013) transformed with pKK, pKK-AzuC, or pKK-$AzuC_{L3STOP}$ or WT or Δ*azuCR* cells expressing GlpD-HA-$His_6$ (GSO1011 and GSO1013, respectively) were grown to an $OD_{600}$ ~0.5 in M63 glycerol medium buffered to pH 7.0 and examined on immunoblots using α-HA antibodies to detect GlpD-HA-$His_6$. The membrane was stained with Ponceau S stain to control for loading. (*C*) MG1655 or Δ*azuCR* (GSO193) cells (top panel) or Δ*azuCR* (GSO193) cells transformed with pKK, pKK-AzuC, or pKK-$AzuC_{L3STOP}$ (bottom panel) were grown in M63 glucose medium to $OD_{600}$ ~1.0 and then washed and resuspended in M63 glycerol medium at pH 5.5. Cells were allowed to grow for an additional 3 h at which point the dehydrogenase activity assay was performed. (*D*) Δ*azuCR* (GSO193) cells (top panel) or Δ*azuCR* Δ*glpD* (GSO1015) cells (bottom panel) transformed with pKK, pKK-AzuC, pKK-AzuC-$I_6L_7$ to $E_6E_7$ or pKK-SpoVM were grown in M63 glucose medium to $OD_{600}$ ~0.5 and then washed and resuspended in M63 glycerol medium at pH 5.5. Cells were allowed to grow for an additional 3 h at which point the dehydrogenase activity assay was performed. For (*C*) and (*D*), lines correspond to the average of three biological replicates with error bars representing one SD.

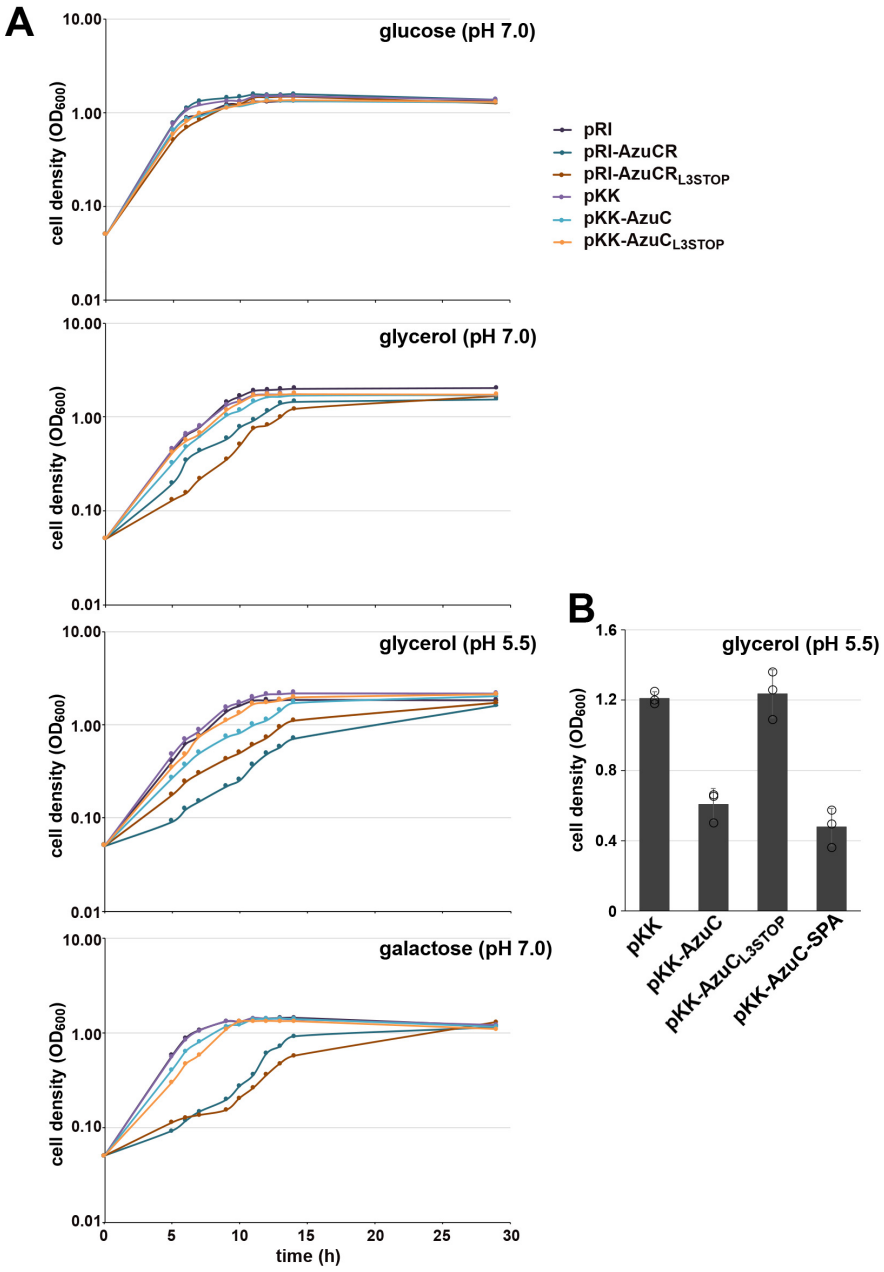


**Fig. S4.** Growth curves for AzuC and AzuCR overexpression. (*A*) Δ*azuCR::kan* strain (GSO193) transformed with pRI, pRI-AzuCR, pRI-AzuCR$_{L3STOP}$, pKK, pKK-AzuC, or pKK-AzuC$_{L3STOP}$ was grown in M63 medium with different carbon sources: glucose (pH 7.0), glycerol (pH 7.0 and 5.5), or galactose (pH 7.0) and growth was tracked by $OD_{600}$ over 30 h. Data for a representative sample are shown. (*B*) Growth of the Δ*azuCR::kan* strain (GSO193) transformed with pKK,

pKK-AzuC, pKK-AzuC$_{L3STOP}$, or pKK-AzuC-SPA in M63 medium with glycerol pH 5.5 was measured at 16 h after dilution by $OD_{600}$. Bars correspond to the average of three biological replicates with circles corresponding to individual data points and error bars representing one SD.

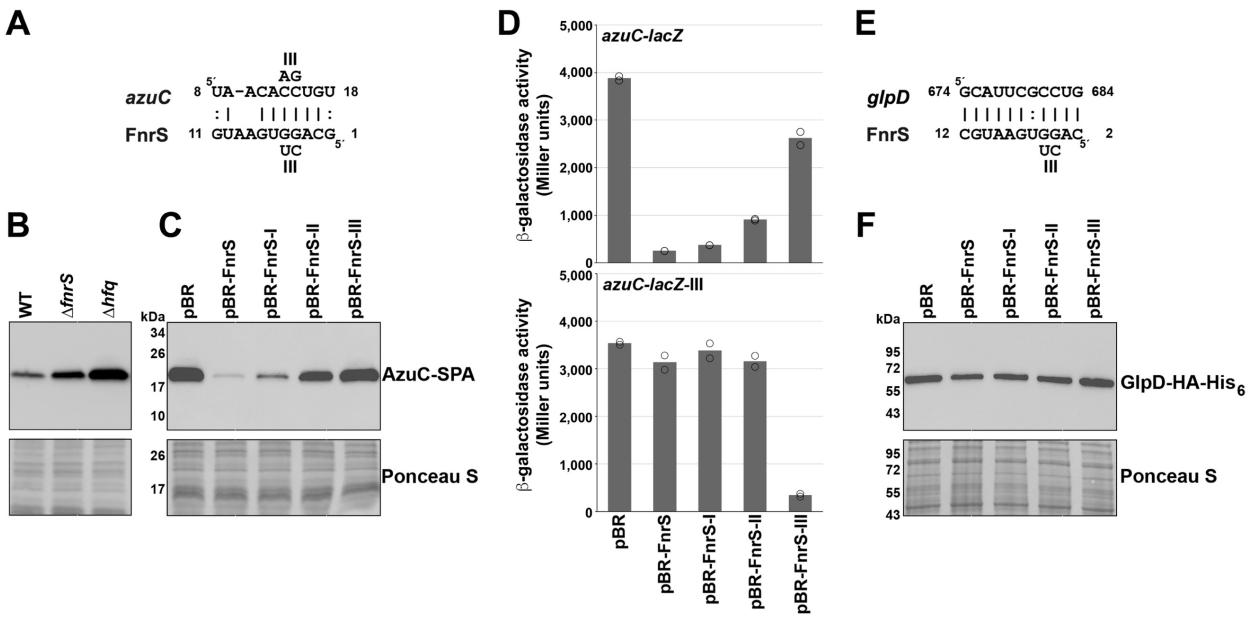


**Fig. S5.** FnrS sRNA represses AzuC and GlpD expression. (*A*) Predicted base pairing between FnrS and AzuCR. The coordinates for both are relative to the +1 of the transcript. (*B*) AzuC-SPA levels in strains lacking Hfq or FnrS. AzuC-SPA levels in WT (GSO351), Δ*hfq* (GSO1007) or Δ*fnrS* (GSO1023) strains grown in LB to $OD_{600}$ ~0.5. (*C*) Effect of FnrS overexpression on AzuC-SPA levels. Cultures of the *azuC-SPA::kan* (GSO351) strain carrying pBR, pBR-FnrS, pBR-FnrS-I, pBR-FnrS-II, or pBR-FnrS-III were grown in LB with 1 mM IPTG to $OD_{600}$ ~0.5. FnrS-I, FnrS-II and FnrS-III have mutations in the three different seed regions involved in base pairing with various mRNA targets as described in (32). For (*B*) and (*C*), α-FLAG antibodies were used to detect the SPA tag, and the membrane was stained with Ponceau S stain to control for loading. (*D*) β-galactosidase activity was assayed for $P_{BAD}$-5′-$UTR_{azuC}$-*lacZ* (GSO1024) or $P_{BAD}$-5′-$UTR_{azuC}$-*lacZ* -III (GSO1025) cells carrying pBR, pBR-FnrS or pBR-FnrS mutants (pBR-FnrS I, II, or III). Cells were grown to $OD_{600}$ ~0.4-0.5 and treated with arabinose (0.2%) and IPTG (1 mM), and cells were grown another 40 min. Bars correspond to the average of two biological replicates with circles corresponding to individual data points. (*E*) Predicted base pairing between FnrS and *glpD*. The predicted region of pairing in *glpD* is within the coding

sequence. The coordinates for FnrS are relative to the +1 of the transcript while the coordinates for *glpD* are relative to the first nucleotide of the start codon. (*F*) Effect of FnrS overexpression on GlpD-HA-$His_6$ levels. Cultures of the *glpD-HA-His*$_6$ (GSO1011) strain carrying pBR, pBR-FnrS, pBR-FnrS-I, pBR-FnrS-II, or pBR-FnrS-III were grown in LB with 1 mM IPTG to $OD_{600}$ ~0.5. α-His antibodies were used to detect GlpD-HA-$His_6$. The average ImageJ signal for the different samples for three independent experiments is as follows: pBR (17,637 ± 358), pBR-FnrS (12,806 ± 2,085), pBR-FnrS-I (11,550 ± 1,459), pBR-FnrS-II (11,976 ± 1,597), or pBR-FnrS-III (18,193 ± 586).