Distinct regions of *H. pylori*’s bactofilin CcmA regulate protein–protein interactions to control helical cell shape

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**Abstract** The helical shape of *Helicobacter pylori* cells promotes robust stomach colonization; however, how the helical shape of *H. pylori* cells is determined is unresolved. Previous work identified helical-cell-shape-promoting protein complexes containing a peptidoglycan-hydrolase (Csd1), a peptidoglycan precursor synthesis enzyme (MurF), a non-enzymatic homolog of Csd1 (Csd2), non-enzymatic transmembrane proteins (Csd5 and Csd7), and a bactofilin (CcmA). Bactofilins are highly conserved, spontaneously polymerizing cytoskeletal bacterial proteins. We sought to understand CcmA’s function in generating the helical shape of *H. pylori* cells. Using CcmA deletion analysis, in vitro polymerization, and in vivo co-immunoprecipitation experiments, we identified that the bactofilin domain and N-terminal region of CcmA are required for helical cell shape and the bactofilin domain of CcmA is sufficient for polymerization and interactions with Csd5 and Csd7. We also found that CcmA’s N-terminal region inhibits interaction with Csd7. Deleting the N-terminal region of CcmA increases CcmA-Csd7 interactions and destabilizes the peptidoglycan-hydrolase Csd1. Using super-resolution microscopy, we found that Csd5 recruits CcmA to the cell envelope and promotes CcmA enrichment at the major helical axis. Thus, CcmA helps organize cell-shape-determining proteins and peptidoglycan synthesis machinery to coordinate cell wall modification and synthesis, promoting the curvature required to build a helical cell.

**Editor’s evaluation**

This study addresses an important unresolved question, the mechanisms governing cell shape in a helical organism. Particularly exciting is the observation that a bactofilin – typically viewed as a cytoskeletal protein – plays a key role in modulating the balance between peptidoglycan synthesis and degradation necessary to generate the helical shape. These findings enhance our understanding of cell wall synthesis in bacteria generally, particularly with regard to the importance of local phenomena for cell shape generation.

**Introduction**

*Helicobacter pylori* is a helical-shaped, Gram-negative bacteria that infects more than half of the world’s population ([Hooi et al., 2017](#)). Chronic infection with *H. pylori* can cause gastric ulcers and cancers; approximately 80% of gastric cancer cases are attributed to *H. pylori* infection ([de Martel et al., 2020](#)). The helical shape of *H. pylori* cells helps establish colonization of the stomach and...
promotes immunopathology during infection (Martínez et al., 2019; Montecucco and Rappuoli, 2001; Sycuro et al., 2010; Sycuro et al., 2012; Yang et al., 2019). Multiple proteins have been identified that are essential for the helical shape of \textit{H. pylori} cells, most of which influence the shape and composition of the peptidoglycan (PG) sacculus directly or indirectly (Sycuro et al., 2010; Sycuro et al., 2013; Sycuro et al., 2012; Yang et al., 2019).

Several cell-shape-determining (Csd) proteins form membrane-spanning protein complexes. Csd5 is a transmembrane protein that binds directly to PG in the periplasm with its SH3 domain and binds to CcmA in the cytoplasm. Csd5 also interacts with ATP synthase and MurF, an essential PG precursor enzyme that catalyzes the final cytoplasmic PG biosynthesis step, independent of interactions with CcmA (Blair et al., 2018; Hrast et al., 2013). Another complex is formed by Csd7, Csd1, and Csd2. Csd7 is a transmembrane protein that stabilizes the PG-hydrolase Csd1, and Csd2, a catalytically inactive homolog of Csd1. Weak interactions between Csd7 and CcmA have been detected by co-immunoprecipitation experiments that include cross-linking reagents followed by mass spectrometry (Yang et al., 2019), but whether CcmA, Csd7, and Csd5 are all part of one protein complex was unclear. CcmA and probes that report on PG synthesis localize to the major helical axis of \textit{H. pylori} cells (regions with a Gaussian curvature value of $5 \pm 1 \mu m^{-2}$ on a helical cell), indicating that CcmA is in the same region of the cell where increased levels of PG synthesis occur (Taylor et al., 2020).

CcmA is the only bactofilin encoded in the \textit{H. pylori} genome. Bactofilins are a class of highly conserved, spontaneously polymerizing, cytoskeletal proteins in bacteria that perform a variety of functions (Bulyha et al., 2013; El Andari et al., 2015; Lin et al., 2017; Osorio-Valeriano et al., 2019) including modulating cell shape in multiple different bacterial species (Brockett et al., 2021; Hay et al., 1999; Jackson et al., 2018; Koch et al., 2011; Sycuro et al., 2010) and morphogenesis of cell appendages (Billini et al., 2019; Caccamo et al., 2020; Kühn et al., 2010), both of which involve modification or synthesis of PG. But how a cytoplasmic bactofilin influences the PG cell wall in the periplasm is unclear. Bactofilins are identified by the presence of a bactofilin domain (DUF583), which forms a $\beta$-helix that is flanked by unstructured terminal regions. The function of the terminal regions and bactofilin domain of bactofilins are debated. It has been proposed that the terminal regions may be involved in modulating polymerization, binding to membranes and/or interacting with other proteins (Deng et al., 2019; Kühn et al., 2010; Vasa et al., 2015). However, experimental evidence to identify the function of the bactofilin domain and terminal regions of CcmA and other bactofilins is lacking.

Here, we performed structure–function analysis to determine how the bactofilin domain and the terminal regions of CcmA contribute to generating the helical shape of \textit{H. pylori} cells by evaluating cell morphology, CcmA polymerization and filament bundling, and interaction with known Csd protein

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**Figure 1.** Predicted structure of CcmA. (A) Sequence alignment between BacA from \textit{C. crescentus} and CcmA from \textit{H. pylori} generated with SWISS-MODEL; gray boxes indicate $\beta$-strands formed in the bactofilin domain, the green box indicates the N-terminal region of CcmA, and the pink box indicates the C-terminal region of CcmA. (B) Model of the bactofilin domain of CcmA generated by RoseTTAFold, which forms a right-handed, three-sided, triangular $\beta$-helix. (C) Five models of CcmA generated in RoseTTAFold displaying the bactofilin domain (gray), N-terminal region (green), and C-terminal region (pink).
interaction partners. Additionally, we identified how protein binding partners impact CcmA localization. We found that interactions between CcmA and other Csd proteins are responsible for CcmA's ability to pattern the helical shape of *H. pylori* cells and identified that interactions between CcmA and its binding partners modulate CcmA localization and modulate protein stability of the PG-hydrolase Csd1. Together, these findings allow us to establish a new model for how *H. pylori*’s helical cell shape is generated. We clarify that CcmA participates in two separate helical-cell-shape-generating protein complexes. Additionally, these studies reveal that bactofilins can influence the protein stability of a PG hydrolase.

**Results**

**CcmA is composed of a bactofilin domain and two short terminal regions**

To facilitate structure–function analysis of CcmA’s biochemical and cell biologic functions, we aligned the amino acid sequence of CcmA with BacA, a well-studied bactofilin from *Caulobacter crescentus* involved in stalk morphogenesis, using SWISS-MODEL (Waterhouse et al., 2018). This alignment revealed that CcmA is composed of a bactofilin domain (DUF 583) surrounded by two short terminal regions (Figure 1A). The N-terminal region is composed of amino acids 1–17, the bactofilin domain is composed of amino acids 18–118, and the C-terminal region is composed of amino acids 119–136. It has been proposed that bactofilins contain a short membrane binding motif within their N-terminal regions, which spans amino acids 3–12 in CcmA (Deng et al., 2019). However, whether the proposed membrane binding motif was functional in CcmA was unknown.

After identifying the boundaries between the bactofilin domain and the terminal regions of CcmA, we used RoseTTAFold (Baek et al., 2021), a protein structure prediction service that uses deep learning-based modeling methods, to predict the structure of CcmA. RoseTTAFold generated five models of CcmA. In all five, the bactofilin domain forms a three-sided, right-handed β-helix with a left-handed twist (Figure 1B), the N-terminal region is unstructured, and part of the C-terminal region forms a short α-helix and caps the end of the bactofilin domain (Figure 1C). In four of the five models, the first several amino acids of the N-terminal region interact with the bactofilin domain, suggesting that the N-terminal region could potentially block a surface of the bactofilin domain.

**The N-terminal region and bactofilin domain are together necessary and sufficient to pattern the helical shape of *H. pylori* cells**

After identifying that CcmA was composed of an N-terminal region that may contain a membrane binding motif, a bactofilin domain, and a C-terminal region, we wanted to identify how each region of CcmA contributed to patterning the helical shape of *H. pylori* cells. The bactofilin literature suggested that the terminal regions on either end of the bactofilin domain may be involved in binding to membranes, modulating protein–protein interactions, or modulating polymerization (Deng et al., 2019; Kühn et al., 2010; Vasa et al., 2015), and we hypothesized deleting the terminal regions of ccmA would alter cell shape. Thus, we generated *H. pylori* strains that have truncated versions of ccmA at the native ccmA locus. We generated five strains, one strain missing the N-terminal region (ΔNT, missing amino acids 2–17), another strain missing the proposed membrane binding motif within the N-terminus (ΔMM, missing amino acids 3–12), a third strain lacking the five amino acids at the end of the N-terminus (Δ13–17, lacking amino acids 13–17), a fourth strain missing the C-terminal region (ΔCT, missing amino acids 119–136), and lastly, a strain expressing only the bactofilin domain that lacks both the N-terminal region and C-terminal region (BD, expressing amino acids 18–118) (Figure 2A). The open-reading frames (ORFs) of the end of csd1 and the beginning of ccmA overlap by 82 nucleotides (Sycuro et al., 2010), thus to avoid impacting the function of Csd1 by removing several amino acids from the C-terminus when truncating the N-terminus of CcmA, we expressed a second copy of csd1 at rdxA, a locus commonly used for complementation (Smeets et al., 2000). In previous work, we confirmed that both ccmA and csd1 can be individually deleted and complemented back without impacting the expression of the other (Sycuro et al., 2010; Yang et al., 2019).

All strains expressed the truncated versions of CcmA measured by Western blotting with an α-CcmA polyclonal antibody; however, in strains lacking the C-terminus (ΔCT and BD), we saw much lower signal on the blot (Figure 2B). In follow-up experiments, we determined that the α-CcmA polyclonal
antibody fails to recognize versions of CcmA lacking the C-terminus (Figure 2-figure supplement 1). We ran a Western blot probed with our polyclonal α-CcmA antibody on purified 6-His WT CcmA and CcmA truncation mutants (Figure 2-figure supplement 2). Although equal concentrations of each purified protein were loaded, the polyclonal α-CcmA antibody poorly recognized versions of CcmA lacking the C-terminal region (ΔCT and BD), suggesting that the ΔCT and BD proteins are likely expressed in H. pylori cells at similar levels to other versions of CcmA but are not robustly recognized by the α-CcmA polyclonal antibody.

Next, after confirming that all truncated versions of CcmA were expressed, we assessed the shapes of the cells and found that strains lacking the N-terminal region (ΔNT and BD) are indistinguishable from ΔccmA cells (Figure 2C and D), while cells lacking the C-terminal region of ccmA resembled WT cells. Cells that lacked either the putative membrane binding motif (ΔMM) or the last five amino acids of the N-terminal region (Δ13–17) were helical but had slightly different side curvature values than the

Figure 2. H. pylori cells expressing CcmA truncation mutants have different cell shapes. (A) Schematic of CcmA and truncation mutants. The N-terminal region (NT, green), bactofilin domain (BD, cyan), and C-terminal region (CT, pink) are indicated, as well as the putative membrane binding motif (MM). (B) Western blot to detect CcmA in whole-cell lysates of H. pylori strains expressing truncated versions of CcmA probed with α-CcmA polyclonal antibody, and α-Cag3 polyclonal antibody as a loading control. (C) Phase-contrast images of H. pylori strains expressing truncated versions of CcmA diagramed in panel (A), scale bars = 1 µm. (D) Histogram displaying the side curvature of H. pylori strains expressing truncated versions of CcmA (WT n = 476 cells, ΔccmA n = 397 cells, ΔN n = 275 cells, ΔMM n = 391 cells, Δ13–17 n = 361 cells, ΔCT n = 263 cells, BD n = 315 cells). Data are representative of two independent biological replicates. Strains used: SSH51A, SSH53A, SSH55A, SSH54A, SSH67A, SSH56B, and SSH57A.

The online version of this article includes the following source data and figure supplement(s) for figure 2:

Source data 1. Raw, unedited Western blots probed with α-Cag3 and α-CcmA antibodies in whole-cell lysates of H. pylori strains expressing truncated versions of CcmA.

Figure supplement 1. Robust CcmA detection with α-CcmA polyclonal antibody requires the C-terminus.

Figure supplement 1—source data 1. Raw, unedited Western blots probed with the α-CcmA antibody in purified CcmA.

Figure supplement 2. Purification of WT and mutant 6-his CcmA.
WT population, suggesting that the N-terminal region of CcmA may have multiple functional regions within it.

**Circular dichroism spectroscopy reveals that CcmA truncation mutants maintain β-strand secondary structure**

Considering the result that *H. pylori* strains lacking the N-terminal region of CcmA, cells resembled ΔccmA mutants, we were curious whether loss of the terminal regions of CcmA might destabilize the protein and/or influence formation of the predicted β-strands (*Figure 1*). Circular dichroism (CD) spectroscopy can be used to identify the secondary structure and folding of proteins. Different structural elements have characteristic CD spectra, and proteins that consist of β-strands have negative peaks at 216 nm ([Greenfield, 2006](https://doi.org/10.7554/eLife.80111)).

We recombinantly expressed and purified 6-his CcmA (*Figure 2—figure supplement 2*) and collected CD spectra from 200 to 260 nm at fixed temperatures to identify the secondary structure and measure its thermal stability.

**Figure 3.** Circular dichroism spectra of purified 6-his CcmA suggests CcmA is composed of predominantly β-sheets. (A) Circular dichroism spectra of WT and truncated versions of CcmA at room temperature (20°C), WT (black), ΔNT (green), ΔCT (pink), and BD (cyan). (B) Circular dichroism spectra of WT CcmA at room temperature (20°C, black), melted (95°C, dotted line), and two point mutants (I55A, red, and L110S, burgundy).

CD spectra of WT CcmA and the truncation mutants ΔNT, ΔCT, and BD at 20°C all look very similar; they have the characteristic negative peak at 216 nm, indicating β-strand secondary structure (*Figure 3A*). Together, these data, combined with the modeling by RoseTTAFold in *Figure 1C*, suggest that CcmA’s bactofilin domain very likely forms a β-helix and confirms that neither terminal region is required for the bactofilin domain to fold properly. In contrast, we also ran CD scans on WT CcmA at 95°C and two CcmA point mutants we have previously shown were unable to rescue the CcmA null cell shape defect, indicating that they were nonfunctional ([Taylor et al., 2020](https://doi.org/10.7554/eLife.80111)) at 20°C. The CD spectra of both mutants (I55A and L110S) at 20°C resemble spectra of melted WT CcmA at 95°C (*Figure 3B*), suggesting that these proteins are not folded. Thus, while CcmA ΔNT, BD, I55A, and L110S all have the same cell shape phenotype when expressed in *H. pylori* – loss of helical cell shape
– the mechanism differs. In CcmA I55A and L110S, CcmA is not folded and nonfunctional, while in CcmA ΔNT and BD, the protein is folded, but loss of the N-terminus causes a loss of helical cell shape.

The bactofilin domain alone is capable of polymerization: the terminal regions of CcmA modulate lateral interactions between CcmA filaments

Given that terminal region truncation mutant proteins appeared stable and displayed CD spectra indicative of a beta helical structure, we wondered whether their cell shape phenotypes correlated with altered polymerization properties. We assessed polymerization by negative stain and transmission electron microscopy (TEM) of purified truncated versions of CcmA and compared those to full-length protein. As previously reported (Holtrup et al., 2019; Taylor et al., 2020), WT CcmA polymerizes into small filaments that measure on average 3.04 nm (SD = 0.79, n = 18) in width. The filaments wrap together to form bundles of approximately 21.34 nm (SD = 4.82, n = 24) wide, and form lattice structures (Figure 4A and G). ΔNT formed filaments and bundles that were more homogenous, straighter, and thinner than WT and appear to only contain 2–4 filaments each. ΔNT failed to form lattice structures (Figure 4B). ΔMM resembled WT CcmA, forming filaments and bundles of approximately the same width as WT and formed lattices (Figure 4C and G). Δ13–17 resembled ΔNT (Figure 4D and G), suggesting that amino acids 13–17 are responsible for filament bundling properties of ΔNT. ΔCT formed bundles that were wider than WT CcmA but failed to form lattices (Figure 4E and G). Lastly, BD formed straight bundles that were narrower than those found in WT samples and failed to form lattices (Figure 4F and G). These data suggest that the bactofilin domain is sufficient for polymerization of CcmA while the N- and C-terminal regions of CcmA regulate interpolymer interactions that impact the width of bundles by modulating the number of filaments that form a bundle, and the ability of CcmA to form lattices. Taken together with our experiments from Figure 2, where we expressed the truncated versions of CcmA in H. pylori, these data suggest that the ability of CcmA to polymerize is not sufficient to pattern the helical shape of H. pylori cells and in vitro lattice formation does not correlate with helical cell shape.

CcmA’s bactofilin domain mediates interactions with Csd5 and Csd7, and the N-terminal region of CcmA inhibits interactions with Csd7

Given that the truncated versions of CcmA were folded properly and were capable of polymerization in vitro, we hypothesized that the loss of helical cell shape phenotypes we observed when we deleted the N-terminal region of CcmA in Figure 2 could be caused by disrupting interactions between CcmA and other cell-shape-determining proteins. CcmA was previously shown to interact with two Csd proteins, Csd5 and Csd7, by co-immunoprecipitation experiments. Csd5-CcmA interactions were readily detected by co-immunoprecipitation followed by mass spectrometry and Western blotting. However, Csd7-CcmA interactions could only be detected when a cross-linking reagent was included in co-immunoprecipitation experiments and mass spectrometry was used to detect CcmA, suggesting that Csd7-CcmA interactions are weak or potentially transient (Blair et al., 2018; Yang et al., 2019). Interactions between Csd5 and Csd7 were never detected by co-immunoprecipitation experiments followed by mass spectrometry in a WT or ΔccmA background (Blair, 2018; Blair et al., 2018; Yang et al., 2019). To probe how each region of CcmA contributes to interactions with Csd5 and Csd7, we performed co-immunoprecipitation experiments followed by Western blotting on strains expressing truncated versions of CcmA. Strains were generated that expressed truncated versions of ccmA at the native ccmA locus, csm1 at the rdxA locus as described earlier, and either csm5-2X-FLAG or csm7-3X-FLAG at their native loci. We immunoprecipitated either Csm5-FLAG or Csm7-FLAG with α-FLAG agarose beads, then performed Western blotting and probed with the α-CcmA polyclonal antibody to identify whether the truncated versions of CcmA could still co-purify. When we immunoprecipitated Csm5-FLAG, all mutant versions of CcmA were detected by Western blotting of the IP fractions, except for the ΔCT mutant (Figure 5A), though it is likely that this mutant can still interact with Csm5-FLAG. Earlier we identified that the C-terminal region of CcmA is required for robust detection by our α-CcmA polyclonal antibody (Figure 2—figure supplement 1), and here, the BD mutant interacts with Csd5. Additionally, we tagged WT CcmA, ΔCT, and BD with a HaloTag in H. pylori at the native ccmA locus and performed a co-immunoprecipitation experiment to detect interactions with Csm5-FLAG (Figure 5—figure supplement 1). We found that both ΔCT-HaloTag and BD-HaloTag were expressed at comparable levels as CcmA-HaloTag and could be pulled down by Csm5-FLAG. These three pieces
Figure 4. The bactofilin domain of CcmA is sufficient for in vitro polymerization while the terminal regions promote lateral polymer interactions. (A–F) Transmission electron microscopy (TEM) of negative stained, purified CcmA, scale bars = 500 nm. White boxes indicate region of micrograph that is enlarged in panel to the right of original. White arrows indicate bundles of CcmA, black arrows indicate lattice structures, and red arrow indicates two bundles wrapping around each other. Data are representative of two independent biological replicates. (G) Box and whiskers plot displaying width of CcmA bundles.
of purified CcmA bundles and filaments. Median, min, max, 25th percentile, and 75th percentile are displayed. One-way ANOVA, Dunnett’s multiple-comparison test. ns p > 0.5, ***p ≤ 0.001, ****p ≤ 0.0001. WT n = 24, BD n = 32.

Figure 4 continued

In Figure 4B, Csd1 levels are lower than in a strain expressing WT CcmA (Figure 6A and B). We performed Western blotting and probed for Csd1 levels in the samples we collected during our Csd7-FLAG co-immunoprecipitation experiments (Figure 5B). We found that in H. pylori strains lacking the N-terminal region of CcmA, Csd7-Csd1 interactions are decreased in comparison to when WT CcmA is expressed (Figure 6C), suggesting that when the N-terminal region of CcmA is deleted CcmA-Csd7 interactions increase and inhibit Csd7 from stabilizing Csd1, causing decreased Csd1 levels. Our data also show that combinations of CcmA and Csd7 and Csd5 to interact, we repeated Csd7-FLAG co-immunoprecipitation experiments in strains expressing WT CcmA, a strain lacking CcmA, and strains expressing CcmA ∆NT or CcmA BD, and probed for interactions with Csd5 by using a polyclonal antibody that recognizes the SH3 domain of Csd5 (Blair et al., 2018). We found that in all the conditions we tested Csd5 never co-purified with Csd7 (Figure 5C). Together, these data suggest that the bactofilin domain of CcmA mediates interaction with Csd5 and Csd7, the N-terminal region of CcmA inhibits interaction with Csd7, and CcmA forms separate complexes with Csd5 and Csd7. Our data also show that versions of CcmA lacking the C-terminal region are expressed at comparable levels to WT CcmA, although they are not recognized as robustly by the α-CcmA polyclonal antibody.

Increased CcmA-Csd7 interactions preclude Csd1-Csd7 interactions, resulting in decreased Csd1 steady-state protein levels

After identifying that Csd1 levels were depleted when the N-terminal region of CcmA was missing, we sought to identify whether Csd1-Csd7 interactions were diminished when Csd7-CcmA interactions increased. We performed Western blotting and probed for Csd1 levels in the samples we collected during our Csd7-FLAG co-immunoprecipitation experiments (Figure 5B). We found that in H. pylori strains lacking the N-terminal region of CcmA, Csd7-Csd1 interactions are decreased in comparison to when WT CcmA is expressed (Figure 6C), suggesting that when the N-terminal region of CcmA is deleted CcmA-Csd7 interactions increase and inhibit Csd7 from stabilizing Csd1, causing decreased Csd1 levels. Our data suggests that Csd1 stability is indirectly regulated by CcmA via Csd7.

CcmA requires binding partners for proper localization to the major helical axis

It has been hypothesized that bactofilins are able to interact with the cell envelope directly and sense specific curvature values (Deng et al., 2019; Kühn et al., 2010; Vasa et al., 2015). To determine whether binding partners are required to direct CcmA to the cell envelope or whether it can localize to the cell envelope on its own, we first created an H. pylori strain that we could use to visualize CcmA by 3D structured illumination microscopy (SIM). We engineered a strain with ccmA-HaloTag as the sole copy of ccmA at the native ccmA locus and a second copy of WT ccmA at rdxA. We found that cells that expressed CcmA-HaloTag as the sole copy of CcmA were still helical but had slightly lower-side curvature than WT cells; adding a second copy of ccmA at rdxA generated cells with side curvature more similar to...
Figure 5. The bactofilin domain interacts with Csd5 and Csd7, and the N-terminal region inhibits Csd7 binding to CcmA. (A, B) Co-immunoprecipitation (co-IP) experiments to probe Csd5-2x-FLAG-CcmA interactions (A) and Csd7-3x-FLAG-CcmA interactions (B) in H. pylori cells are shown. Top row: Ponceau S staining of input fractions. Second row: Western blot probed with α-CcmA polyclonal antibody of input fractions. Third row: Western blot probed with α-FLAG monoclonal antibody of IP fractions. Bottom row: Western blot probed with α-CcmA polyclonal antibody of IP fractions. (C) Co-IP experiments to probe Csd7-3x-FLAG interactions with CcmA and Csd5 in H. pylori strains with and without CcmA. Top row: Ponceau S staining of input fractions. Second row: Western blot probed with α-SH3 polyclonal antibody of input fractions to detect the SH3 domain of Csd5. Third row: Western blot probed with α-CcmA polyclonal antibody of input fractions. Fourth row: Western blot probed with α-FLAG monoclonal antibody of IP fractions to detect Csd7-3x-FLAG. Fifth row: Western blot probed with α-SH3 polyclonal antibody to detect the SH3 domain of Csd5 in IP fractions. Bottom row: Western blot probed with α-SH3 polyclonal antibody to detect the SH3 domain of Csd5 in IP fractions.

Figure 5 continued on next page
WT cells (Figure 7—figure supplement 1A and B). Next, to identify whether CcmA is capable of localizing to the cell envelope at its preferred curvature alone or whether localization is mediated by a binding partner, we deleted csd5 and csd7 individually and together in our ccmA-HaloTag strain. As an additional control, we deleted csd6 in the ccmA-HaloTag strain. Csd6 is a PG carboxypeptidase that does not appear to interact with CcmA, Csd5, or Csd7 (Blair et al., 2018; Sycuro et al., 2013; Yang et al., 2019). Similar to Δcsd5 mutants, Δcsd6 mutants show a straight rod shape phenotype. We fixed and permeabilized cells, then labeled CcmA-HaloTag with a fluorescent ligand (JF-549) that binds to HaloTag and counterstained with fluorescent wheat germ agglutinin (WGA), which binds to PG, to label the cell envelope. We collected images of hundreds of cells from each population using 3D SIM and used software previously described to generate 3D reconstructions of individual cells from the WGA channel and calculate the Gaussian curvature at each point on the cell surface. Then we performed analysis to identify how much CcmA-HaloTag signal was at the cell envelope versus in the center of cells, and curvature enrichment analysis to investigate whether CcmA-HaloTag is enriched or depleted at specific Gaussian curvature values (Bratton et al., 2019; Taylor et al., 2020).

In WT cells, we found that CcmA-HaloTag signal is present as foci and small arcs that colocalize with the cell envelope (white signal) and there were some foci in the center of the cell (Figure 7A, top row). The CcmA-HaloTag signal closely resembled what we saw when we visualized CcmA-FLAG by immunofluorescence in previous work (Taylor et al., 2020). In cells lacking csd6, we found that CcmA-HaloTag signal is present at the cell envelope and there are some foci in the center of cells (Figure 7A, second row). In cells lacking csd7, cells are slightly curved and CcmA-HaloTag signal colocalizes with the cell envelope; however, there are also some foci in the center of the cell (Figure 7A, third row). In cells lacking csd5, there appears to be more CcmA-HaloTag signal in the center of cells than associated with the cell envelope (Figure 7A, fourth row). In cells lacking both csd5 and csd7, we found that the cell shape resembles a Δcsd7 mutant; however, there appears to be more CcmA-HaloTag signal in the center of the cells than in the Δcsd7 mutant, similar to the Δcsd5 mutant (Figure 7A, bottom row).

To quantify how much CcmA-HaloTag signal was associated with the cell envelope as compared to the amount of CcmA-HaloTag found in the center of the cells, we developed a method to measure the amount of CcmA-HaloTag signal that colocalized with the cell surface as we computationally generated sequential cell surface shells with smaller or larger diameters (Figure 7B, Figure 7—figure supplement 1C and D). We used the fluorescent WGA channel that labels the PG cell wall to generate the cell surface. As we computationally shrink the cell surface (offset from surface decreases), we observe the relative concentration of WGA and CcmA-HaloTag signal with respect to the position of the cell surface. In both a WT and Δcsd5 cell, the WGA signal peaks at offset from surface = 0 nm where the PG cell wall is located within the periplasm. In WT cells, CcmA-HaloTag signal peaks near ~50 nm from the PG cell wall and declines further to the inside of the cell. Based on cryo-electron tomography data, an approximate distance of 50 nm from the PG cell wall to the cytoplasm in H. pylori is expected (Asmar et al., 2017; Chang et al., 2018; Kühn et al., 2010; Lu et al., 2022; Qin et al., 2017). In contrast, in a Δcsd5 cell, the CcmA-HaloTag signal peaks near ~100 nm from the cell surface and continues to be high even as the surface of interest approaches center of the cell (approximately ~300 nm), indicating that more CcmA-HaloTag signal is found near the center of the cell in Δcsd5 cells than in WT cells. When we performed the same analysis on the entire populations of cells (292 WT and 326 Δcsd5), we found that while the overall trends were the same as when we analyzed individual cells, there was a lot of variability in the WT cell population. The variability of the WT population arises because many WT cells have both a population of CcmA-HaloTag at the cell...
envelope (peak at ~50 nm) and in the center of the cell that causes a broader left tail in the population-level data (Figure 7—figure supplement 1C and D). In contrast, the Δcsd5 population-level signal peak is broader and shifted towards the center of the cell (~80 nm). Thus, Csd5 is required for CcmA to localize to the cell envelope.

To identify where CcmA-HaloTag signal that is at the cell surface is in relation to cell curvature, we calculated the Gaussian curvature values of all cell surfaces represented in the populations. WT cells (which are helical) have a larger range of Gaussian curvature values on their cell surfaces (Figure 7C).
Figure 7. Csd5 is required for CcmA-HaloTag to localize to the cell envelope at the major helical axis. (A) Maximum intensity projections (MIPs) and frames from Z-stacks of H. pylori cells labeled with wheat germ agglutinin (WGA) to label the cell wall (magenta) and JF549 ligand to label CcmA-HaloTag (green), pixels are white where the two signals colocalize. Green arrows indicate cytoplasmic CcmA-HaloTag. White arrows indicate CcmA-HaloTag signal that colocalizes with the cell envelope. Scale bars = 1 µm. (B) Representative plots displaying the relative concentration of CcmA-HaloTag signal that colocalizes with the cell envelope. Scale bars = 1 µm. (B) Representative plots displaying the relative concentration of CcmA-HaloTag signal that colocalizes with the cell envelope. Scale bars = 1 µm. (B) Representative plots displaying the relative concentration of CcmA-HaloTag signal that colocalizes with the cell envelope. Scale bars = 1 µm. (B) Representative plots displaying the relative concentration of CcmA-HaloTag signal that colocalizes with the cell envelope. Scale bars = 1 µm.
HaloTag and WGA signal from one WT and one Δcsd5 cell at the cell surface (offset from surface = 0) and at computationally generated cell surfaces that are inside (offset from surface < 0) and outside (offset from surface > 0) of the cell surface. (C) Representative H. pylori cells from each population with Gaussian curvature values mapped on the cell surface. (D, E) Top: histogram of Gaussian curvature of cell surfaces of each population. Bottom: surface Gaussian curvature enrichment of relative concentration of CcmA signal in each population where 1 = uniform concentration of CcmA, shaded with Gaussian curvature values mapped on the cell surface. (D) Top: histogram of Gaussian curvature of cell surfaces of each population. Bottom: surface Gaussian curvature enrichment of relative concentration of CcmA signal in each population where 1 = uniform concentration of CcmA, shaded with Gaussian curvature values mapped on the cell surface. (E) Top: histogram of Gaussian curvature of cell surfaces of each population. Bottom: surface Gaussian curvature enrichment of relative concentration of CcmA signal in each population where 1 = uniform concentration of CcmA, shaded with Gaussian curvature values mapped on the cell surface.

Figure supplement 1. Cell shape of CcmA-HaloTag strains and analysis of CcmA-HaloTag surface signal in populations.

Figure supplement 2. Cell shape and Csd1 levels of ccmA ΔNT Δcsd5 mutants.

Figure supplement 2—source data 1. Raw, unedited Western blots probed with α-Cag3 and α-Csd1 antibodies in whole-cell lysates of H. pylori strains.

and D) compared to all other shape mutants. Interestingly, although all shape mutants are not helical, they do display different surface Gaussian curvature distributions and Δcsd5 and Δcsd5/7 populations look nearly identical, suggesting that Δcsd5/7 double mutants resemble the shape of a Δcsd7 mutant.

In WT cells, CcmA-HaloTag is enriched at the major helical axis (Gaussian curvature value of 5 ± 1 µm⁻²; Taylor et al., 2020); enrichment peaks at +3.35 µm⁻² to +5.31 µm⁻² Gaussian curvature and is depleted at Gaussian curvature values lower than +0.97 µm⁻² and higher than +6.67 µm⁻². These data are consistent with previous experiments where CcmA-FLAG was detected by immunofluorescence (Taylor et al., 2020). In contrast, in all other strains, CcmA-HaloTag is depleted at regions of Gaussian curvature where CcmA-HaloTag is enriched in WT cells (+3.35 µm⁻² to +5.31 µm⁻²), although those Gaussian curvature values are still present in all cell-shape-mutant populations (Figure 7C D). In Δcsd6 and Δcsd7 mutants, CcmA-HaloTag is enriched at negative Gaussian curvature values. In Δcsd6 mutants, enrichment peaks at and continues to be enriched at all values lower than +0.11 µm⁻² Gaussian curvature (to −4 µm⁻² Gaussian curvature). In Δcsd7 mutants, CcmA-HaloTag enrichment peaks at +0.15 µm⁻² and is enriched at all values below +0.15 µm⁻² (to −5.4 µm⁻² Gaussian curvature; Figure 7E). In contrast to Δcsd6 and Δcsd7 mutants, in Δcsd5 mutants, CcmA-HaloTag is depleted at negative Gaussian curvature values (Figure 7E). Interestingly, in a Δcsd5/7 double mutant, the CcmA-HaloTag enrichment pattern mirrors the Δcsd5 mutant enrichment pattern. In Δcsd5 mutants, CcmA-HaloTag is enriched at Gaussian curvature values from −1.18 µm⁻² to +2.5 µm⁻² and has a peak enrichment at +0.68 µm⁻², CcmA-HaloTag is depleted at all other values of Gaussian curvature. In a Δcsd5/7 double mutant, CcmA-HaloTag is enriched at Gaussian curvatures of −0.99 µm⁻² to +3.46 µm⁻² and peaks at +0.85 µm⁻², CcmA-HaloTag is depleted at all other values of Gaussian curvature. Taken together, these data suggest that Csd5 directs CcmA to the cell envelope; CcmA is unable to localize to the cell envelope without Csd5. When helical cell shape is disrupted but Csd5 is present (in a Δcsd6 or Δcsd7 strain), CcmA localizes to regions of negative Gaussian curvature instead of the major helical axis. The finding that in a Δcsd5/Δcsd7 double mutant the CcmA-HaloTag localization resembles a Δcsd5 mutant indicates that Csd5, not Csd7, is the primary driver of CcmA localization in WT cells.

Given the requirement for Csd5 to localize CcmA to the cell envelope, we wondered what would happen in a Δcsd5ccmAΔNT strain. We could envisioned two possibilities. (1) Without Csd5, CcmA ΔNT would not be able to localize to the inner membrane and be close enough to interact with Csd7, thus CcmA ΔNT and Csd7 would not interact at high levels. In this case, Csd1 should be stable and the shape phenotype would resemble Δcsd5 cells (straight rod). (2) Even though Csd5 is not present to direct CcmA ΔNT to the inner membrane, interactions between CcmA ΔNT and Csd7 are strong enough to direct CcmA to the inner membrane, thus CcmA ΔNT and Csd7 would still interact at high levels. In this case, we would expect diminished Csd1 protein levels and a cell shape phenotype similar to a Δcsd5Δcsd1 (curved rod; Sycuro et al., 2012) and similar to Δcsd5Δcsd7 shown in Figure 7. As shown in Figure 7—figure supplement 2, the Δcsd5ccmAΔNT strain has a curved rod phenotype and low levels of Csd1 supporting the second possibility, further reinforcing our hypothesis that Csd5 and Csd7 are part of separate protein complexes.
**Discussion**

Multiple proteins have been identified that are required to generate the helical shape of *H. pylori* cells, several of which form protein complexes. However, the function of many of the individual proteins and complexes was unknown. Here, we sought to understand the function of the bactofilin CcmA in generating the helical shape of *H. pylori* cells. We identified the function of each of the regions/domains of CcmA in patterning the helical shape of *H. pylori* cells and clarified how the cell-shape-determining protein complexes in *H. pylori* function. This work provides mechanistic insight into how CcmA, and potentially other bactofilins, regulates cell shape and morphogenesis of bacteria.

In *H. pylori*, when the cytoplasmic bactofilin CcmA is lost, cells lose their helical shape and the PG muropeptide composition is altered ([Sycuro et al., 2010](#)). The PG sacculus is what ultimately controls the shape of bacterial cells, and it has been hypothesized that modification of PG cross-linking generates curvature and twist of the sacculus ([Huang et al., 2008; Sycuro et al., 2010](#)), but how loss of the cytoplasmic bactofilin CcmA could change the PG sacculus (in the periplasm) was unknown. Previously, we showed that CcmA and Csd7 (which is required for Csd1 protein stability) interact weakly and that ΔccmA, Δcsd1, Δcsd2, and Δcsd7 mutants all share the same curved rod cell shape phenotype and PG muropeptide composition ([Sycuro et al., 2010; Yang et al., 2019](#)). These observations led us to hypothesize that CcmA may indirectly regulate Csd1 activity and thus the PG cell wall, but a mechanism linking CcmA and Csd1 was unknown. In past work, we also identified that CcmA interacts with the transmembrane protein Csd5, which binds directly to PG, providing another potential link between CcmA and the PG sacculus. Additionally, we discovered that CcmA localizes to the major helical axis of *H. pylori* cells where rates of PG synthesis are also increased, suggesting that CcmA regulates PG synthesis in some fashion ([Blair et al., 2018; Taylor et al., 2020; Yang et al., 2019](#)). In this study, we built upon our previous body of work and identified several new mechanisms that clarify how CcmA regulates the helical cell shape, and thus the PG sacculus, of *H. pylori* by modulating interactions with Csd5 and Csd7.

We began this study by identifying how the bactofilin domain and short terminal regions of CcmA contributed to CcmA’s ability to pattern helical cell shape in *H. pylori*. First, we identified that the N-terminal region of CcmA and the bactofilin domain are required for helical cell shape, but that the C-terminal region was not required. Next, in co-immunoprecipitation experiments, we found that the bactofilin domain of CcmA interacts with Csd5 and Csd7 and the N-terminal region inhibits the bactofilin domain from interacting with Csd7. When the N-terminal region of CcmA is removed, the bactofilin domain of CcmA binds to Csd7, which prevents Csd7 from stabilizing Csd1, causing Csd1 depletion. This provides the first potential mechanism by which CcmA regulates the PG hydrolase Csd1. Interestingly, when we modeled CcmA, in 4/5 models the N-terminal region of CcmA binds to the bactofilin domain of CcmA, potentially blocking one of the surfaces ([Figure 1C](#)). It is possible that by binding to the bactofilin domain the N-terminal region prevents interactions between the bactofilin domain of CcmA and Csd7. Future experiments will be needed to further clarify this mechanism.

We found that when other cell-shape-determining genes are deleted, CcmA can no longer localize to the major helical axis of the cell (Gaussian curvature value of 5 ± 1 µm⁻²; [Taylor et al., 2020](#)), suggesting that CcmA is directed to its preferred location by a binding partner and is not capable of localizing to or sensing cell envelope curvature on its own. Loss of Csd5 causes substantially less CcmA to localize to the cell envelope, instead CcmA accumulates in the center of cells. The CcmA signal that does colocalize with the cell envelope in Δcsd5 and Δcsd5/7 cells is enriched near zero Gaussian curvature, which is the most common sidewall curvature value in these cells, and is depleted at all other Gaussian curvature values. When csd6 or csd7 are deleted, CcmA localizes to regions of negative Gaussian curvature. These data suggest that Csd5 recruits CcmA to the cell envelope. If helical cell shape is disrupted (due to loss of another cell shape protein) while Csd5 is still present, Csd5 directs CcmA to regions of negative Gaussian curvature. Additionally, the finding that CcmA localization phenotypes are the same in a Δcsd5 mutant and a Δcsd5/7 double mutant suggest that Csd5, not Csd7, controls CcmA localization. These results also support the hypothesis that there are two helical-cell-shape-generating protein complexes, one containing Csd5 and one containing Csd7.

Using what we have learned in this study, we can build a new model of the protein complexes that pattern the helical shape of *H. pylori* cells ([Figure 8](#)). In one protein complex, CcmA’s bactofilin domain interacts with the cytoplasmic N-terminal region of the transmembrane protein Csd5 directing CcmA to the cell envelope. Csd5 brings MurF and PG precursors to the same region of the cell envelope...
Figure 8. Schematic depicting CcmA's role in the helical cell shape complexes. There are two protein complexes, one containing Csd5, MurF, and CcmA, and another containing Csd7, Csd1, and Csd2. (A) In WT cells, Csd5 recruits WT CcmA to the cell envelope via CcmA's bactofilin domain. The N-terminal region of CcmA inhibits interaction with Csd7, allowing Csd1 to function and excluding Csd1 from the CcmA-Csd5-MurF complex. (B) In CcmA \( \Delta N \)-terminal region, CcmA & Csd7 interact, and Csd1/2 are destabilized. (C) \( \Delta \)csd5 cells lack Csd5, allowing CcmA to be present in the cytoplasm.
loss of the N-terminal region (residues 1–10 in Ttbac) generated smaller, more
Thermus thermophilus
data correlate with what has been previously reported in the literature; in the bactofilin Ttbac from
domain is capable of polymerizing on its own, the N-terminal region promotes lateral interactions
the hydrophobic effect (Taylor et al., 2020);
hydrophobic core of the proteins and mutating these residues destabilizes the proteins likely through
Vasa and colleagues in BacA (V75 and F130 in BacA) and are predicted to reside in the interior of the
function are unfolded (Figure 3). The I55 and L110 residues are homologous to residues mutated by
Figure 3
to fold. Additionally, we identified that two previously studied CcmA point mutants that cause loss of

terminated CcmA does not impact the ability of the bactofilin domain
β-
data show that the bactofilin domain is a
- helix and the two short terminal regions are largely unstruc-
tured. Deletion of the terminal regions of CcmA does not impact the ability of the bactofilin domain
to fold. Additionally, we identified that two previously studied CcmA point mutants that cause loss of
function are unfolded (Figure 3). The I55 and L110 residues are homologous to residues mutated by
Vasa and colleagues in BacA (V75 and F130 in BacA) and are predicted to reside in the interior of the
hydrophobic core of the proteins and mutating these residues destabilizes the proteins likely through
the hydrophobic effect (Taylor et al., 2020; Vasa et al., 2015). We also identified that the bactofilin
domain is capable of polymerizing on its own, the N-terminal region promotes lateral interactions
between filaments, and the N- and C-terminal regions are both necessary for lattice formation. These
data correlate with what has been previously reported in the literature; in the bactofilin Ttbac from
Thermus thermophilus loss of the N-terminal region (residues 1–10 in Ttbac) generated smaller, more
ordered bundles of filaments (Deng et al., 2019), and it has been previously reported that purified
∆CT CcmA from H. pylori does not form lattice structures (Holtrup et al., 2019). Interestingly, in the
case of CcmA ∆NT and BD, both purified proteins readily polymerize in vitro, yet are unable to pattern
the helical cell shape of H. pylori when expressed in vivo, suggesting that CcmA has other functions
besides being a purely structural element. For CcmA to function properly in the H. pylori cell-shape-
determining protein complexes, it must polymerize, interact with Csd5, and inhibit interaction with
Csd7. However, it is unclear what state CcmA is in when it interacts with Csd5 and inhibits interaction
with Csd7; whether CcmA is polymerized or monomeric when it is in complex is unknown and will
require further investigation.

In this study, we identified several putative functional motifs within CcmA. The N-terminal region
(amino acids 1–17) is important for patterning helical cell shape and regulates interactions with Csd7,
suggesting that the N-terminal region is a functional motif. Our data also suggest that amino acids
13–17 in the N-terminal region of CcmA promote lateral interactions between filaments in vitro and
may be another functional motif. However, in CcmA, the putative membrane-binding motif (amino
acids 3–12) suggested by Deng and colleagues (Deng et al., 2019) did not show a clear function in
any of the experiments we conducted. In H. pylori, the putative membrane-binding motif of CcmA
likely does not direct the protein to the inner membrane. Instead, interaction with Csd5 is required for
CcmA to localize to the inner membrane. Interestingly, the C-terminal region of CcmA is not required
for cell shape or interactions with Csd5 or Csd7 interactions, but it does have a role in regulating
polymerization properties of purified CcmA.

Figure 8 continued

cells expressing CcmA ∆NT, Csd5 recruits ∆NT CcmA to the cell envelope via CcmA’s bactofilin domain. The
bactofilin domain of CcmA now also binds to Csd7 in a separate complex, inhibiting Csd7 from stabilizing Csd1.
(C) When Csd5 is absent, CcmA cannot localize to the cell envelope, MurF is not directed to a particular location.
OM, outer membrane; IM, inner membrane. This figure was created using Biorender.com.
Bactofilins are commonly found as part of complexes in other bacteria and help spatially organize cells to regulate where certain functions occur (Sichel and Salama, 2020; Surovtsev and Jacobs-Wagner, 2018). Our findings regarding how CcmA functions within a complex in H. pylori provide insight into how bactofilins operate within complexes in other bacterial species. Mechanisms similar to how CcmA functions in H. pylori have been reported in other systems; in A. biprostecum, a PG hydrolase, SpmA, directs the bactofilin BacA to the site of stalk synthesis, then BacA organizes the rest of the complex (Caccamo et al., 2020) to control stalk morphogenesis. Here, we identified that CcmA is not capable of localizing to the cell envelope without Csd5. While Csd5 is unique to the Helicobacter genus, transmembrane proteins in other species may also be essential for localization of bactofilins. Interestingly, the genomic arrangement of csd1 (an M23 metallopeptidase) and CcmA overlapping is conserved in several other curved and helical species of Campylobacterota and Pseudomonadota (Billini et al., 2019; Caccamo et al., 2020; Sycuro et al., 2010), and has been reported in a spirochete (Jackson et al., 2018), suggesting that bactofilins may regulate M23 metallopeptidases to promote PG remodeling at specific regions of the cell envelope to control cell shape and morphogenesis of other morphological features. Our work is the first to suggest a mechanism by which a bactofilin can regulate a PG hydrolase indirectly through a transmembrane membrane protein and protein stability.

### Materials and methods

#### Key resources table

| Reagent type (species) or resource | Designation                                      | Source or reference                      | Identifiers             | Additional information          |
|-----------------------------------|--------------------------------------------------|------------------------------------------|-------------------------|---------------------------------|
| Antibody                          | Monoclonal α-FLAG M2 antibody produced in mouse | Sigma                                    | Cat# F1804; RRID:AB_262044 | Used at 1:5000 for Western blot |
| Antibody                          | Polyclonal rabbit α-CcmA                         | Blair et al., 2018                      |                         | Used at 1:10,000 for Western blot |
| Antibody                          | Polyclonal rabbit α-SH3                         | Blair et al., 2018                      |                         | Used at 1:5000 for Western blot |
| Antibody                          | Monoclonal mouse α-HaloTag                       | Promega                                 | Cat# G9211; RRID:AB_2688011 | Used at 1:10,000 for Western blot |
| Antibody                          | Polyclonal rabbit α-Csd1                         | Yang et al., 2019                       |                         | Used at 1:10,000 for Western blot |
| Antibody                          | Polyclonal rabbit α-Cag3                         | Pinto-Santini and Salama, 2009          |                         | Used at 1:20,000 for Western blot |
| Chemical compound, drug           | α-FLAG M2 Affinity Gel                           | MilliporeSigma                          | Cat# A2220               |                                  |
| Chemical compound, drug           | Janelia Fluor HaloTag ligand 549 (JF-549)        | Promega                                 | Cat# GA1110              |                                  |
| Chemical compound, drug           | Wheat germ agglutinin, Alexa Fluor 488 Conjugate | Invitrogen                              | Cat# W11261              |                                  |
| Other                             | ProLong Diamond Antifade Mountant                | Invitrogen                              | Cat# P36961              | Mountant used for fluorescent microscopy |
| Commercial assay or kit           | In-Fusion HD cloning kit                         | Takara                                  | Cat# 638920              |                                  |
| Commercial assay or kit           | Zero Blunt TOPO PCR Cloning Kit, with One Shot Top10 Chemically Competent E. coli cells | Thermo Fisher Scientific                | Cat# K280020             |                                  |

#### Bacterial strain construction and growth

Strains used in this work, as well as primers and plasmids used in strain construction, are available in Tables 1–4. H. pylori was grown in Brucella Broth (BD) supplemented with 10% heat-inactivated fetal bovine serum (Cytiva HyClone) without antimicrobials or on horse blood (HB) agar plates with microbials as described (Sycuro et al., 2010). For resistance marker selection, HB agar plates were supplemented with 15 μg/ml chloramphenicol, 25 μg/ml kanamycin, or 60 mg/ml sucrose. H. pylori strains were grown at 37°C under micro-aerobic conditions in a tri-gas incubator (10% CO₂, 10% O₂, and 80% N₂). For plasmid selection and maintenance in Escherichia coli, cultures were grown in Lysogeny broth (LB) or on LB-agar at 37°C supplemented with 100 μg/ml ampicillin or 25 μg/ml kanamycin.
| Strain   | Relevant genotype or description                                      | Reference or source       |
|----------|-----------------------------------------------------------------------|----------------------------|
| DCY26    | csd7::catsacB                                                         | Yang et al., 2019         |
| DCY28    | csd7::cat                                                             | Yang et al., 2019         |
| DCY71    | csd7-3X-FLAG                                                          | Yang et al., 2019         |
| DCY77    | csd7-3x-FLAG, ccmA::cat                                               | Yang et al., 2019         |
| JS02     | ccmA::ccmA-40 aa linker-HaloTag, rdxA::ccmA, csd5::cat2kan            | This work                 |
| JS09     | ccmA::ccmA-40 aa linker-HaloTag, rdxA::ccmA, csd5::cat2kan, csd7::cat | This work                 |
| JTH1     | csd5-2X-FLAG cat                                                      | Blair et al., 2018        |
| JTH3     | ccmA-2X-FLAG cat                                                      | Blair et al., 2018        |
| KBH157   | csm5-2X-FLAG, mrf-3X-VSV-G::cat                                       | Blair et al., 2018        |
| LSH100   | Wild-type H. pylori, NSH57 with fliM repaired                          | Sycuro et al., 2010       |
| LSH108   | rdxA::kansacB                                                         | Sycuro et al., 2010       |
| LSH113   | csd1::cat                                                             | Sycuro et al., 2010       |
| LSH117   | ccmA::catsacB                                                         | Sycuro et al., 2010       |
| LSH121   | csd1::cat, rdxA::csd1                                                 | Sycuro et al., 2010       |
| LSH123   | csd5::cat                                                             | Sycuro et al., 2010       |
| LSH148   | ccmA::catsacB, rdxA::ccmA                                             | Sycuro et al., 2010       |
| SSH31C   | ccmA::ccmA-12 aa linker-HaloTag                                       | This work                 |
| SSH33    | ccmA::ccmA-40 aa linker-mNeonGreen                                    | This work                 |
| SSH38A   | ccmA::ccmA-40 aa linker-HaloTag                                       | This work                 |
| SSH39B   | ccmA::ccmA-40 aa linker-HaloTag, rdxA::ccmA                           | This work                 |
| SSH41A   | ccmA::ccmA-3X-FLAG                                                   | This work                 |
| SSH49A   | ccmA::ccmA-40 aa linker-HaloTag, rdxA::ccmA, csd5::cat                | This work                 |
| SSH50A   | ccmA::ccmA-40 aa linker-HaloTag, rdxA::ccmA, csd7::catsacB            | This work                 |
| SSH51A   | rdxA::csd1                                                            | This work                 |
| SSH53A   | ccmA::ccmA-12 aa linker-HaloTag                                       | This work                 |
| SSH54A   | ccmA::ccmA ΔMM, rdxA::csd1                                             | This work                 |
| SSH55A   | ccmA::ccmA ΔNT, rdxA::csd1                                             | This work                 |
| SSH56B   | ccmA::ccmA ΔCT, rdxA::csd1                                             | This work                 |
| SSH57A   | ccmA::ccmA BD, rdxA::csd1                                             | This work                 |
| SSH58A   | ccmA::ccmA ΔMM, rdxA::csd1, csd5::ccmA-2X-FLAG-cat                     | This work                 |
| SSH59A   | ccmA::ccmA ΔNT, rdxA::csd1, csd5::ccmA-2X-FLAG-cat                     | This work                 |
| SSH60A   | ccmA::ccmA ΔCT, rdxA::csd1, csd5::ccmA-2X-FLAG-cat                     | This work                 |
| SSH65A   | ccmA::ccmA BD, rdxA::csd1, csd5::ccmA-2X-FLAG-cat                      | This work                 |
| SSH67A   | ccmA::ccmA Δ13–17, rdxA::csd1                                         | This work                 |
| SSH68A   | ccmA::ccmA Δ13–17, rdxA::csd1, csd5::ccmA-2X-FLAG-cat                  | This work                 |
| SSH70A   | ccmA::ccmA-40 aa linker-HaloTag, rdxA::ccmA, csd6::cat                 | This work                 |
| SSH78B   | ccmA::ccmA ΔMM, rdxA::csd1, csd7::ccmA-3X-FLAG                         | This work                 |
| SSH79A   | ccmA::ccmA ΔNT, rdxA::csd1, csd7::ccmA-3X-FLAG                         | This work                 |
| SSH80A   | ccmA::ccmA ΔCT, rdxA::csd1, csd7::ccmA-3X-FLAG                         | This work                 |

Table 1 continued on next page
All genetic manipulations of *H. pylori* were performed on the chromosome via natural transformation and allelic exchange by homologous recombination of either purified PCR products or plasmids. We used chloramphenicol (cat) and kanamycin (aphA3) resistance cassettes as markers in some strains (*Trieu-Cuot et al., 1985; Wang and Taylor, 1990*). In others, we used selectable and counter-selectable cassettes catsacB (chloramphenicol resistance and sucrose susceptibility) and kansacB (kanamycin resistance and sucrose susceptibility) for constructing marker-less strains (*Copass et al., 1997*).

All genomic DNA preparations from *H. pylori* were performed with the Wizard Genomic DNA Purification Kit (Promega). All plasmids were purified with the QIAprep Spin Miniprep Kit (QIAGEN).

**Construction of H. pylori strains expressing truncated versions of ccmA**

To construct strains expressing truncated versions of CcmA, we first generated a parent strain expressing an extra copy of csd1 at rdxA, a locus commonly used for complementation (*Smeets et al., 2000*). First, we transformed LSH108 (rdxA::kansacB) with plasmid pLKS31 (csd1 in pRdxA) to replace kansacB at rdxA with csd1, then selected for colonies that grew on HB agar plates (*Humbert and Salama, 2008*) containing sucrose but could not grow on HB plates containing kanamycin to generate strain SSH51A. Then, we replaced the ccmA gene with a catsacB cassette so that marker-less truncated versions of ccmA could be engineered by allelic exchange. To do this, we transformed strain SSH51A with genomic DNA from LSH117 (ccmA::catsacB) and selected for colonies that grew on HB plates containing chloramphenicol to generate strain SSH53A. We transformed strain SSH53A with purified PCR products generated by PCR SOEing (*Horton, 1995*) that had truncated versions of ccmA flanked by 800 nucleotides of homology upstream of the ccmA and 500 nucleotides of homology downstream of the ccmA, and selected for colonies that grew on HB plates containing sucrose, but not on HB plates containing chloramphenicol. After confirming that strains grew on selective plates, strains were confirmed by PCR and Sanger sequencing to sequence the rdxA and ccmA loci.

To generate the ∆NT strain (SSH55A, lacking amino acids 2–17), we used primers ‘Csd1 F’ and ‘remove aa 2–17 R’ to amplify the upstream PCR product and primers ‘remove aa 2–17 F’ and ‘CcmA SDM dn R’ to amplify the downstream PCR product from LSH100 (WT) genomic DNA. The resulting products were purified using the QIAquick PCR Purification Kit (QIAGEN). Then we stitched them together with PCR SOEing (*Horton, 1995*), gel extracted the product using the QIAquick Gel Extraction Kit (QIAGEN), and used the product directly for natural transformation of SSH53A. To generate the ∆MM strain (SSH54A, lacking amino acids 3–12), the same strategy was used, but we used primers ‘remove aa 3–12 F’ and ‘remove aa 3–12 R’ instead of ‘remove aa 2–17 F’ and ‘remove aa 2–17 R.’ To generate Δ13–17 strain (SSH67A, lacking amino acids 13–17), the same strategy was...
used, but with primers ‘remove aa 13–17 2 F’ and ‘remove aa 13–17 2 R.’ To generate the ΔCT strain (SSH56B, lacking amino acids 119–136), we followed the same strategy but used primers ‘remove aa 119–136 F’ and ‘remove aa 119–136 R.’

To generate the BD strain (SSH57A, only amino acids 18–188), we amplified genomic DNA from strain SSH55A by PCR with primers ‘Csd1 F’ and ‘remove aa 119–136 R’ and genomic DNA from SSH56B with primers ‘CcmA SDM dn R’ and ‘remove aa 119–136 F’ and purified them using the QIAquick PCR Purification Kit (QIAGEN). Then we stitched them together with PCR SOEing (Horton, 1995), gel extracted the product using the QIAquick Gel Extraction Kit (QIAGEN), and used the product directly for natural transformation of SSH53A.

To generate strain SSH109A, a strain lacking csd5 and the N-terminus of ccmA, strain SSH55A (ccmA::ccmA ∆NT, rdxA::csd1) was transformed with genomic DNA from LSH123 (csd5::cat). Colonies that grew on HB plates containing chloramphenicol were selected.

**Construction of *H. pylori* strains expressing FLAG-tagged versions of Csd5 or Csd7**

To construct strains expressing Csd5-2X FLAG, strains with truncated versions of ccmA (SSH54A, SSH55A, SSH56B, SSH57A, SSH67A) were transformed with genomic DNA from JTH1 (contains a cat cassette downstream of csd5-2X FLAG at the native csd5 locus). Colonies that grew on HB plates containing chloramphenicol were selected and confirmed by PCR. These are strains SSH58A, SSH59A, SSH60A, SSH65A, and SSH68A.

To construct strains expressing Csd7-3X FLAG, DCY71 (Csd7-3X-FLAG) was transformed with genomic DNA from LSH117 to replace ccmA with catsacB and clones that grew on HB plates supplemented with chloramphenicol were selected. Next, strains were transformed with genomic DNA from strains SSH54A, SSH55A, SSH56B, SSH57A, or SSH67A to replace catsacB with truncated versions of ccmA, clones that grew on HB plates supplemented with sucrose but not chloramphenicol were selected. Then, strains were transformed with genomic DNA from SSH51A to replace kansacB with csd1 at rdxA locus, clones that grew on HB plates supplemented with kanamycin were selected. Finally, clones were transformed with genomic DNA from SSH51A to replace kansacB with csd1 at rdxA, clones that grew on HB plates supplemented with sucrose but not on HB.
### Table 4. Primers used in this work.

| Name | Sequence 5′ to 3′ |
|------|------------------|
| **Construction of *H. pylori* strains** | |
| Csd1 F | GAGTCGTACATTAATGTGCATATCT |
| CcmA SDM dn R | GCTCATTGTAGTGTTGGAT |
| Remove aa 2–17 F | CAATAAAGAAAGAGACATAGATGGGCGACTATCATC GCTC |
| Remove aa 2–17 R | GAGGCGATGATGTCGCCATCTGATGCTCCTTTTTT |
| Remove aa 3–12 F | AATAAAGAAAGAGACATAGATGGCAGCAAAACAG G |
| Remove aa 3–12 R | CCGTTTTTTTCTGCCATCTGTGCTCCTTTTTTATTT |
| Remove aa 119–136 F | GGGAAACTGCGCTCCTAAGATAGGGAATGTCCAAT CTAG |
| Remove aa 119–136 R | CTAGATTGATACCTTTCTAATCCCTAGGGCGAGTT TCCC |
| Remove aa 13–17 2 F | ATAACAATAAATACGGCTAATGCGACTATCATCG CTCA |
| Remove aa 13–17 2 R | TGGCGGATGATGTCGCCATTAGCCGGATTATTTATTTT |
| CcmA_40aa_linker_upstream_R | ACCTTTGTCCGCTACCTCAAGTATTTTTTTAAATTTT |
| CcmA_mNeonGreen_dnstrm_F | GATGGTAGAAATTATAATAATAGGGAATGTCCA ATCTAGT |
| CcmA_12_aa_link_Halo_R | AGTACCGATTTCACCACCCCACCATCCACACCACACCAC |
| CcmA_Halo_dnstrm_R | AGACTAGATTGATACCTTTCTAATCCCGGAAATCTCC AGA |
| CcmA_12_aa_link_Halo_F | ATTTGAATAGGCTGTGAGTTGGGTGGTAGT |
| 40 aa linker halotag R | GGTGTTGTTGTAATGTGAAATTCGCTGATAGT |
| 40 aa linker halotag F | TTAAAAAGTTGATGCTGTGAGTTGGGTGGTAGT |
| C1_828 | GATAAGATTGAAAAAGTGGAT |
| C1_829 | TTATCGATCCCACAAACTGG |
| **Cloning for protein purification** | |
| CcmA_pET15b_BamHI | GTTAGCAGCGCGGATATCCCTATTTCTTATTTTCTT |
| pET15b_ccmA_delta_aa2to17_Ndel | CGCGCGCGCGCAACATGATGCGACTATCATCGCTC AAG |
| CcmA_pET15b_BamHI | GTTAGCAGCGCGGATATCCCTATTTCTTATTTTCTT |
| pET15b_ccmA_delta_aa3to12_Ndel | CGCGCGCGCGCAACATGATGCGACTATCATCGCTC |
| CcmA_pET15b_Ndel | CGCGCGCGCGCAACATGATGCGACTATCATCGCTC |
| pET15b_ccmA_delta_aa119to136_BamHI | GTTAGCAGCGCGGATATCCCTATTTCTTATTTTCTT |
| pET15b_ccmA_delta_aa13to17_Ndel | CGCGCGCGCGCAACATGATGCGACTATCATCGCTC |

*Table 4 continued on next page*
plates supplemented with kanamycin were selected and confirmed by PCR and Sanger sequencing of the \textit{rdxA} and \textit{ccmA} loci. Confirmed strains are SSH78B, SSH79A, SSH80A, SSH81B, and SSH82.

**Construction of \textit{H. pylori} strains expressing CcmA-HaloTag**

We used a flexible 40 amino-acid-long linker to link a HaloTag to the C-terminus of CcmA. To do this, we first constructed a strain where the C-terminus of \textit{ccmA} was fused to \textit{mNeonGreen} with the 40 amino-acid-long linker between the two, then modified it to replace \textit{mNeonGreen} with \textit{HaloTag}. We used a gBlock (IDT) that contained the linker followed by a codon-optimized version of \textit{mNeonGreen} and used PCR SOEing (Horton, 1995) to generate a construct that had \textit{ccmA-linker-mNeonGreen} flanked with 800 nucleotides of homology upstream of the \textit{ccmA} and 500 nucleotides of homology downstream of the \textit{ccmA}. We amplified the upstream portion of the construct from LSH100 (WT) genomic DNA with primers ‘Csd1 F’ and ‘CcmA-40aa_linker_upstream_R’ and the downstream portion with primers ‘CcmA-linker-mNeonGreen_dnstrm_R’ and ‘CcmA SDM dn R.’ After cleaning up the PCR products with QIAquick PCR Purification Kit (QIAGEN), we performed PCR SOEing (Horton, 1995) to stitch together the two PCR products and the gBlock, the product was gel extracted using QIAquick Gel Extraction Kit (QIAGEN), then TOPO cloned using the Zero Blunt TOPO PCR Cloning Kit (Invitrogen). The plasmid was then used as a template to transform LSH117 for allelic exchange to replace the \textit{catsacB} cassette at the \textit{ccmA} locus with the construct. Clones that grew on HB plates containing sucrose but not chloramphenicol were selected and confirmed by PCR and Sanger sequencing to generate strain SSH33.

Next, we constructed a strain where HaloTag was fused to the C-terminus of \textit{ccmA} with a 12-amino-acid-long linker. To do this, we generated a construct containing \textit{ccmA-12aa-linker-HaloTag} flanked by 800 nucleotides of homology upstream of the \textit{ccmA} and 500 nucleotides of homology downstream of the \textit{ccmA}. We amplified PCR products from genomic DNA from LSH100 with primers ‘Csd1 F’ and ‘CcmA-12aa_link_HaloR’ and primers ‘CcmA_Halo_dnstrm_R’ and ‘CcmA SDM dn R.’ Next, we amplified a PCR product containing HaloTag from plasmid pFC30K (Promega) with primers ‘CcmA-12aa_link_Halo_F’ and ‘CcmA_Halo_dnstrm_R.’ After cleaning up the PCR products with QiAquick PCR Purification Kit (QIAGEN), we performed PCR SOEing (Horton, 1995) to stitch the three PCR products together and TOPO cloned the purified PCR product using the Zero Blunt TOPO PCR Cloning Kit (Invitrogen). The plasmid was then used as a template to transform LSH117 for allelic exchange to replace the \textit{catsacB} cassette at the \textit{ccmA} locus with the construct. Clones that grew on HB plates containing sucrose but not chloramphenicol were selected and confirmed by PCR and Sanger sequencing to generate strain SSH31C.

Finally, to construct a strain expressing \textit{ccmA-40 aa-linker-HaloTag} at the native \textit{ccmA} locus, we amplified a PCR product with primers ‘Csd1 F’ and ‘40 aa linker halotag R’ from SSH33 genomic DNA and another PCR product with primers ‘40 aa linker halotag F’ and ‘CcmA SDM dn R’ from SSH31C genomic DNA. After cleaning up the PCR products with QiAquick PCR Purification Kit (QIAGEN), we
performed PCR SOEing (Horton, 1995) to stitch the two products together, then gel extracted using QIAquick Gel Extraction Kit (QIAGEN), and TOPO cloned using the Zero Blunt TOPO PCR Cloning Kit (Invitrogen). The plasmid was then used to transform LSH117 and replace the catsacB cassette at the ccmA locus with the construct. Clones that grew on HB plates containing sucrose but not chloramphenicol were selected and confirmed by PCR and Sanger sequencing (SSH38A). We noticed that these strains had altered morphology (expanded view Figure 4), so to adjust the cell shape to be more like WT, we added a second copy of WT ccmA at rdxA by transforming LSH148 (ccmA:: catsacB, rdxA::ccmA) with genomic DNA from SSH38A to replace catsacB cassette with ccmA-40 aa linker- HaloTag to generate strain SSH39B. Clones that grew on HB plates containing sucrose but not chloramphenicol were selected and confirmed by PCR.

To generate strains lacking csd5, csd7, and csd6, SSH39B was transformed with genomic DNA from either LSH123 (csd5::cat), DCY26 (csd7::cat), or TSH17 (csd6::cat). Clones that grew on HB plates supplemented with chloramphenicol were selected and confirmed by PCR.

Construction of vectors for recombinant expression of 6-his CcmA
Truncated versions of CcmA were cloned into pET15b, a cloning vector used for expressing N-terminal 6-his tagged proteins, with the In-Fusion cloning kit (Takara). First, pET15b was linearized with restriction enzymes BamHI-HF (NEB) and NdeI (NEB), then gel extracted and purified using QIAquick Gel Extraction Kit (QIAGEN). Next, PCR products containing truncated versions of CcmA flanked by 15 nucleotides of homology to the plasmid backbone were amplified and the products were purified with the QIAquick PCR Purification Kit (QIAGEN).

The ∆NT product was amplified from SSH55A genomic DNA with primers ‘CcmA-pET15b_BamH1′ and ‘pET15b_ccmA_delta_aa2to17_NdeI.’ The ∆MM product was amplified from SSH54A genomic DNA with primers ‘CcmA-pET15b_BamH1′ and ‘pET15b_ccmA_delta_aa3to12_NdeI.’ The A13–17 product was amplified from SSH67A genomic DNA with primers ‘CcmA-pET15b_BamH1′ and ‘pET15b_ccmA_delta_aa13to17_NdeI.’ The ∆CT product was amplified from SSH56B genomic DNA with primers ‘CcmA-pET15b_NdeI’ and ‘pET15b_ccmA_delta_aa119to136_BamHI.’ The BD product was amplified from SSH57A genomic DNA with primers ‘pET15b_ccmA_delta_aa2to17_NdeI’ and ‘pET15b_ccmA_delta_aa119to136_BamHI.’

After cloning following the manufacturer’s instructions, colonies were verified by restriction digest and Sanger sequencing. Confirmed plasmids were transformed into BL21 DE3 (NEB) for recombinant expression of 6-his-CcmA.

Protein structure prediction
SWISS-MODEL was used to identify the boundaries between the bactofilin domain and the terminal regions of CcmA. The primary amino acid sequence of CcmA from the H. pylori G27 genome (GenBank: CP001173.1) was submitted to SWISS-MODEL (https://swissmodel.expasy.org; Waterhouse et al., 2018) where the structure of BacA from C. crescentus (Vasa et al., 2015) was used to model CcmA. RoseTTAFold was used to predict the structure of CcmA; the same primary amino acid sequence of CcmA was submitted to the Robetta protein prediction service (https://robetta.bakerlab.org; Baek et al., 2021). Models generated in RoseTTAFold were visualized using PyMOL (The PyMOL Molecular Graphics System, version 2.4.2 Schrödinger, LLC).

Western blotting
Whole-cell extracts of H. pylori were prepared by harvesting 1.0 optical density at 600 nm (OD600) of log phase liquid culture (0.3–0.7 OD600) by centrifugation for 2 min. Then, bacteria were resuspended
to a concentration of 10 OD₆₀₀ per ml with 2× protein sample buffer (0.1 M Tris–HCl, 4% SDS, 0.2% bромophenol blue, 20% glycerol) with or without 5% β-mercaptoethanol (BME). Samples were loaded onto Mini-PROTEAN TGX 4–15% polyacrylamide gels (Bio-Rad) and transferred onto polyvinylidene difluoride (PVDF) membranes using the Trans-Blot TurboTransfer System (Bio-Rad) system. Membranes were either stained with 0.1% Ponceau S solution in 5% acetic acid (Sigma) or immediately blocked for 2 hr at room temperature in 5% non-fat milk in Tris-buffered saline containing 0.05% Tween-20 (TBS-T), then incubated either overnight at 4°C or at room temperature for 1 hr with primary antibodies, 1:10,000 for α-CcmA (Blair et al., 2018), 1:5000 for α-FLAG (M2, Sigma), 1:10,000 for α-Csd1 (Yang et al., 2019), 1:20,000 for α-cag3 (Pinto-Santini and Salama, 2009), 1:5000 for α-SH3 (Blair et al., 2018), or 1:10,000 for α-HaloTag (Promega). Then, it was washed five times with TBS-T over a 30 min period, incubated with secondary antibodies for 1 hr at room temperature at 1:20,000 with appropriate horseradish peroxidase-conjugated α-immunoglobulin antibodies (α-rabbit for CcmA, Csd1, SH3, or Cag3; α-mouse for FLAG and HaloTag, Santa Cruz Biotechnology), and then washed five times with TBS-T over a 30 min period. Proteins were detected using a chemiluminescent substrate according to the manufacturer’s instructions (Pierce ECL substrate for standard Western blotting or Immobilon Western HRP substrate [Millipore] for Western blotting following co-immunoprecipitation experiments) and imaged directly with the Bio-Rad gel documentation system. Some membranes were stripped and re-probed with different antibodies, in that case membranes were first rehydrated in TBS-T then incubated in low pH stripping buffer for 20 min while shaking at room temperature (200 mM glycine, 3.5 mM SDS, 1% Tween-20, pH 2.2). After stripping, membranes were washed three times with TBS-T for 5 min each before proceeding to the blocking step.

Western blot densitometry
Western blot densitometry was performed using Image Lab software version 6.0.1 (Bio-Rad). The lanes on each Western blot were defined, then the adjusted total lane volume was calculated by the software. To calculate relative Csd1 levels, the signal detected by the α-Csd1 antibody for each sample was divided by the signal detected by the α-Cag3 antibody from the same sample on the same membrane. After calculating relative Csd1 levels, they were normalized by dividing the signal by the relative Csd1 level from the SSH51A strain (rdxA::csd1).

Phase-contrast microscopy and quantitative morphology analysis
Phase-contrast microscopy and image analysis was performed as previously described (Sycuro et al., 2010). Briefly, cells were grown in liquid culture to mid-log phase (0.3–0.7 OD₆₀₀), centrifuged, and resuspended in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) and mounted on glass slides. Cells were imaged with a Nikon TE 200 microscope equipped with a ×100 oil immersion lens objective and Photometrics CoolSNAP HQ charge-coupled-device camera. Images were thresholded with Fiji (Schindelin et al., 2012) and CellTool software (Pincus and Theriot, 2007) was used to measure both side curvature and central axis length.

Protein purification
Purification of 6-His CcmA was performed exactly as described previously (Taylor et al., 2020). E. coli strains used to recombinantly express CcmA were pKB62A in BL21 DE3, pSS15A in BL21 DE3, pSS16A in BL21 DE3, pSS17A in BL21 DE3, pSS18B in BL21 DE3, pSS19A in BL21 DE3, pKB69H in BL21 DE3, and pKB72D in BL21 DE3.

Circular dichroism
After purification of 6-his CcmA, samples were dialyzed against 10 mM CAPS pH 11 at a volume of 1:10,000 for 24 hr, then the buffer was changed, and dialysis continued for another 24 hr. After dialysis was complete, samples were normalized to 0.5 mg/ml. CD spectra were collected from 180 to 260 nm while holding the temperature constant with a Jasco J-815 Circular Dichroism Spectropolarimeter.

Negative stain and TEM
After purification of 6-his CcmA, samples were dialyzed against 25 mM Tris pH 8 for 24 hr. Samples were then normalized to 1 mg/ml and placed on carbon-coated grids after glow-discharging. Samples were fixed in ½ X Karnovsky’s fixative and washed with 0.1 M cacodylate buffer and water, then
stained with Nano-W (Nanoprobes) for 60 s, wicked, then stained for another 60 s. Images were acquired on a kV Thermo Fisher Talos 120C LaB₆ microscope equipped with a Ceta Camera using Leginon software (Suloway et al., 2005). The width of bundles and filaments was measured using Fiji (Schindelin et al., 2012).

Co-immunoprecipitation experiments
Co-immunoprecipitation experiments were performed as previously described in Blair et al., 2018 and Yang et al., 2019 with slight modifications. H. pylori expressing FLAG tags fused to either Csd5 or Csd7 were grown to mid-log phase in liquid culture and 15 OD₆₀₀ were harvested by centrifugation at 10,000 RPM for 10 min at 4°C. Cells pellets were resuspended in 1.8 ml of cold lysis buffer (20 mM Tris pH 8, 150 mM NaCl, 2% glycerol, 1% Triton X-100, and EDTA-free protease inhibitor [Pierce]). The cells were sonicated at 10% power for 10 s intervals with a microtip (Sonic Dismembrator Model 500, Branson) until visibly cleared, then centrifuged at 20,000 RCF for 30 min at 4°C. The soluble fraction was then incubated with 40 µl beads (α-FLAG M2 agarose beads [Sigma]) or α-VSV-G agarose beads (Sigma) equilibrated in wash buffer (20 mM Tris pH 8, 150 mM NaCl, 2% glycerol, 0.1% Triton X-100) for 90 min at 4°C. The beads were then washed four times in 10 ml cold wash buffer, and samples were centrifuged at 2000 RPM for 2 min between washes. Beads were then boiled in 45 µl 2× sample buffer without BME for 10 min and subjected to Western blotting.

Labeling with fluorescent WGA and HaloTag ligands
H. pylori expressing CcmA-HaloTag were grown to mid-log phase and fixed with 4% PFA for 45 min at room temperature. After fixing, bacteria were permeabilized for 1 hr at room temperature with PBS + 0.05% Triton X-100. Bacteria were then resuspended in PBS and centrifuged onto #1.5 coverslips. Next, coverslips were incubated on a droplet of PBS containing 30 µg/ml af488-conjugated WGA (Invitrogen) and 4 µM JF-549 (Promega) for 30 min at room temperature. Coverslips were washed four times (10 min each) by placing on fresh droplets of PBS + 0.1% Tween-20, then mounted on glass slides with ProLong Diamond Antifade Mountant (Invitrogen).

3D structured illumination microscopy
3D SIM was performed on a DeltaVision OMX SR equipped with PCO scientific CMOS cameras, 488 nm and 568 nm lasers, and an Olympus ×60/1.42 U PLAN APO oil objective with oil matched for the sample refractive index. The DeltaVision OMX SR has a resolution of approximately 100 nm in the X and Y directions and 300 nm in Z. 512 × 512 pixel Z-stack images were acquired with 125 nm spacing and 3 µm total thickness. Figures were generated by changing channel colors, adjusting brightness and contrast in Fiji, and brightness and contrast settings were maintained across the entire image (Schindelin et al., 2012). Figures were assembled in Adobe Illustrator.

3D reconstructions and curvature enrichment analysis
3D reconstructions were generated, and curvature CcmA enrichment analysis was performed exactly as described in Taylor et al., 2020 using existing software (Bratton et al., 2019; Bratton et al., 2018). Briefly, 3D SIM Z-stack images were used to generate a 3D triangular meshwork surface with roughly 30 nm precision from the WGA channel. After generating the 3D triangular meshwork, the Gaussian curvature was calculated at every location on the surface of the cell, and we performed curvature enrichment analysis to identify which GC values CcmA signal was associated with where uniform signal = 1. Because the absolute amount of CcmA signal can differ between cells and because the illumination throughout the field is nonuniform, we set the average CcmA signal on each cell as 1. Then we measured each cell’s curvature-dependent CcmA signal intensity relative to 1, normalized by the amount of the curvature present on that individual cell surface.

Analysis of intensity of CcmA-HaloTag signal at cell surface and within cells
A custom MATLAB script was used to compute the relative intensity of CcmA-HaloTag signal at the cell surface and at surfaces generated by shrinking the surface of interest toward the centerline of the cell (negative offsets) or increasing the surface away from the centerline (positive offsets). For each of these shells, or surface offsets, we calculated the average WGA signal and the average CcmA-HaloTag
signal. To account for errors in spatial alignment between the two color channels, before calculating the intensity at each shell, we performed a rigid body alignment procedure to maximize the intensity of the signal contained within the nominal reconstructed surface (0 nm offset). Consistent with the fact that we utilized the WGA channel for shape reconstructions, this alignment algorithm returned offsets of less than one pixel (one pixel = 80 nm) for the WGA channel. The CcmA-HaloTag channel showed more variability in the offsets, sometimes requiring an offset of almost 200 nm along the focal direction, consistent with small, wavelength-dependent variations in focal position. After calculating the average intensity at each subshell, or resized surface, we normalized the highest concentration to 1 before averaging across all the cells in our sample.

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Author contributions

Sophie R Sichel, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Writing – original draft, Writing – review and editing; Benjamin P Bratton, Data curation, Software,
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**Additional files**

**Supplementary files**

- MDAR checklist

**Data availability**

Data generated or analysed during this study are included in the manuscript and supporting file; Source Data files have been provided for Figures 2, 5, 6 and 7. Microscopy data are available at BioImage Archive and accession code is S-BIAD462.

The following dataset was generated:

| Author(s)       | Year | Dataset title                                                                 | Dataset URL                                                                 | Database and Identifier |
|-----------------|------|-------------------------------------------------------------------------------|----------------------------------------------------------------------------|-------------------------|
| Sichel SR       | 2022 | Microscopy data generated in the study "Distinct regions of H. pylori's bactofilin regulate protein interactions to control cell shape" | [https://www.ebi.ac.uk/biostudies/BioImages/studies/S-BIAD462](https://www.ebi.ac.uk/biostudies/BioImages/studies/S-BIAD462) | BioImage Archive, S-BIAD462 |

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