The flat ribbon configuration of the periplasmic flagella of *Borrelia burgdorferi* and its relationship to motility and morphology

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ABSTRACT

Electron cryotomography was used to analyze the structure of the Lyme disease spirochete *Borrelia burgdorferi*. This methodology offers a new means for studying the native architecture of bacteria by eliminating the chemical fixing, dehydration, and staining steps of conventional electron microscopy. Using electron cryotomography, we noted membrane blebs formed at the ends of the cells. These blebs may be precursors to vesicles that are released from cells grown *in vivo* and *in vitro*. We found that the periplasmic space of *B. burgdorferi* was quite narrow (16.0 nm) compared that of *Escherichia coli* and *Pseudomonas aeruginosa*. However, in the vicinity of the periplasmic flagella, this space is considerably widened (42.3 nm). In contrast to previous results, the periplasmic flagella did not form a bundle, but rather a tight-fitting ribbon that wraps around the protoplasmic cell cylinder in a right-handed sense. We show how the ribbon configuration of the assembled periplasmic flagella is optimal for both swimming and forming the flat-wave morphology. Previous results indicate that *B. burgdorferi* motility is dependent on the rotation of the periplasmic flagella in generating backward-moving waves along the length of the cell. This swimming requires that the rotation of the flagella exerts force on the cell cylinder. Accordingly, a ribbon is clearly more beneficial than a bundle, as this configuration allows each periplasmic flagellum to have direct contact with the cell cylinder in order to exert that force.
INTRODUCTION

Spirochetes are a monophyltic phylum that have a unique morphology (7,34). These bacteria have a protoplasmic cell cylinder, which includes the plasma membrane and peptidoglycan layer, and an outer membrane. The region between the plasma membrane and the outer membrane constitutes the periplasmic space. The periplasmic flagella, which are subterminally attached to the ends of the protoplasmic cell cylinder, reside in this space. A given periplasmic flagellum is attached at only one end, extends toward the center of the cell, and is rotated by a basal motor anchored to the protoplasmic cell cylinder. The periplasmic flagella at each end form a group of filaments, and depending on the species, each group contains from one to hundreds of periplasmic flagella.

The motility of *Borrelia burgdorferi*, the Lyme disease spirochete, is quite complex (see (9,24,25) for recent reviews on spirochete motility). This species is capable of swimming in both low-viscosity media and also in viscous gel-like media that inhibit the motility of most other bacteria (18,22). A typical *B. burgdorferi* cell runs, stops, flexes (pauses and forms a distorted shape) and reverses direction. Several lines of evidence indicate that during a run, the two groups of 7-11 periplasmic flagella rotate asymmetrically, i.e. one group rotates clockwise (CW), and the other rotates counter-clockwise (CCW) (9,23,31). (As a frame of reference, a given flagellum is viewed from its end along the filament towards its insertion into the protoplasmic cell cylinder.) A cell in the flexing mode is thought to have its groups of periplasmic flagella rotating in the same direction, i.e. both rotate CW, or both rotate CCW (31). During a running interval, the cell has a flat-wave appearance, with waves of constant amplitude being propagated from its anterior to the posterior end (18). Rotation of the groups of the two groups of PFs in
opposite directions generates backward-moving waves along the cell body that propel the cell forward (9,17,18,24). Video 1 at http://www.uic.edu/orgs/blast. B. burgdorferi has many motility and chemotaxis genes in common with those of rod-shaped bacteria (15,24); it is chemotactic to many compounds including glucosamine, N-acetyl glucosamine, and glutamate (1). However, the paradigm for spirochete chemotaxis is notably different from that of other bacteria such as Escherichia coli and Salmonella enterica serovar Typhimurium (9). For example, B. burgdorferi rotates its groups of periplasmic flagella asymmetrically during a run (9,23), whereas for E. coli and S. enterica serovar Typhiumurium, all the flagella rotate CCW during the run (43). In addition, although CheY and CheA homologs are involved in B. burgdorferi chemotaxis (1,23,31), the nature of the signal that coordinates rotation of the two groups of periplasmic flagella is unknown (9).

The complex geometry of B. burgdorferi is beginning to be understood. B. burgdorferi cells are approximately 10 to 20 µm long and 0.33 µm in diameter (17,18). The periplasmic flagella attached to one end of the cell are long enough to overlap with those of the other end (19). Because the cell cylinder is rod-shaped in mutants that lack the periplasmic flagella, and regain the flat-wave morphology in genetically complemented strains that regain the periplasmic flagella, these organelles are concluded to have a skeletal function (30,40). Purified periplasmic flagella are tightly coiled left-handed helices with most having a helix pitch of 1.48 µm and a helix diameter of 0.28 µm (10). In addition, the periplasmic flagella undergo a helical transformation as a function of pH as found with many flagella of other bacteria (S. Satoshi, S.I. Aizawa, Md. Motaleb, and N. W. Charon, unpublished). In high-voltage electron micrographs of intact cells, the periplasmic flagella appear as a left-handed helical bundle with a helix pitch equal to the cell’s wavelength (17). Although the bundle wraps around the cell cylinder in a right-handed sense, along the cell axis it is left-handed. Because the shape of the isolated periplasmic
flagella and cell cylinders are so markedly different from that seen in the intact cells, they evidently exert force on one another to influence each other's shape. Recent experiments and calculations using elasticity theory, coupled with measurements of the mechanical properties of purified periplasmic flagella and protoplasmic cell cylinders employing laser tweezers, indicate that the flat wave cell morphology is a natural consequence of the interaction of helical periplasmic flagella and the rod-shaped cell cylinder (C. Dombrowski, W. Kan, M. A. Motaleb, N.W. Charon, R. E. Goldstein, and C. W. Wolgemuth, submitted for publication).

Cryoelectron microscopy, and electron cryotomography, also referred to as cryoelectron tomography, offers a new methodology for studying the architecture of bacteria (21,29,44,46). Previous electron-microscopic analysis used specimens of cells that were chemically fixed and stained; this methodology has been shown to introduce artifacts. For example, electron microscopy of hydrated sections of E. coli and Pseudomonas aeruginosa indicated that the periplasmic space is markedly thinner than results previously obtained using fixed cells (27). Here we analyzed the structure of B. burgdorferi using electron cryotomography, and compared our results to those recently reported for Treponema primitia and Treponema denticola (20,33). We found that not only is its periplasmic space quite thin, but that the periplasmic flagella do not form a bundle as previously thought (17,30). Instead, the periplasmic flagella assemble into a very tightly packed flat ribbon that also widens the periplasmic space in the domain where they reside. Furthermore, we show how the ribbon configuration of the assembled periplasmic flagella is optimal for both swimming and forming the flat-wave morphology. (This research was presented in part at the Bacterial Locomotion and Sensory Transduction Meeting, January, 2007, Laughin, NV).


MATERIALS AND METHODS

Strains, culture conditions, and sample preparation. The high passage B. burgdorferi strain B31A was used for all analyses (5). Cells were grown in BSK complete medium (Sigma-Aldrich) at 34°C in an atmosphere of 3.0% CO₂ (30). To prepare cells for electron cryotomography, approximately 1.5 ml of late logarithmic phase cells were centrifuged at 1,200 x g for five minutes in a microcentrifuge at room temperature. Approximately 1.4 ml of the supernatant fluid was discarded, and the cell pellet was gently resuspended by pipetting up and down in the remaining 100 µl and then cooled in ice. To prepare grids, first a thin carbon coat was evaporated onto Quantifoil electron microscopy specimen grids (R3.5/1, Quantifoil Microtools, Jena, Germany). Immediately, the grids were treated with a 10 nm colloidal gold solution to provide fiducial markers for alignment of the tomographic tilt series (35). Approximately 5 µl of the cell suspension was applied to the grid without dilution or washing. The excess medium was blotted with filter paper. The grid was immediately plunge-frozen into liquid ethane (12), and stored under liquid nitrogen for future examination.

Electron microscopy and tomographic reconstruction. Images were recorded at -178°C, using a JEOL JEM4000FX equipped with a Gatan GIF2002 energy filter. The microscope was operated at 400 kV acceleration voltage in zero-loss energy-filtered mode. Single-axis tilt series were collected with a 1° increment and 120° angular range. The thickness of the ice layer was 300-400 nm, as measured by electron energy-loss spectroscopy (13). The total electron dose for a tilt series was 70-90 e/Å², with the higher dose used with thicker specimens. The calculated resolution in the x-y plane was 8 nm. The calculated z (depth) resolution was 12 nm, due to the elongation factor from the “missing wedge” caused by the limited tilt range (36).
underfocus value, 15 µm, was chosen to maximize the transfer of information at the expected
resolution limit, in order to optimize the signal-to-noise ratio (28). All image processing was
done using SPIDER (14), and the reconstructions were computed by weighted back-projection.
Isosurface models were traced on WACOM Cintiq 20WSX tablet (Wacom Co. Saitama, Japan)
using AMIRA software (Mercury Computer Systems, Chelmsford, MA). To confirm chirality
determinations, previously embedded Leptonema illini (fomerly Leptospira illini), which is a
known right-handed helical spirochete, was analyzed in the same manner as frozen B.

burgdorferi cells (8,17).

RESULTS

Overall cell morphology. Electron cryotomography was used to analyze B. burgdorferi cells.
Cells were propagated in growth medium, concentrated by centrifugation, dropped onto electron
microscopy grids with a support film perforated with small holes, and immediately plunged into
liquid ethane. Because the spirochetes are flexible, distortion of a cell occurred at the edges of
the holes of the support film. The analyses we report are exclusively on those regions of the cells
which fell across the holes. B. burgdorferi cells are considerably longer than the diameter of the
grid holes. Consequently, only a relatively small region of the cell (2-3 µm, which is less than 25
% of the cell length) was analyzed in a given tomogram.

The cells in general had a flat-wave appearance as seen in living or fixed cells. The large size of
the spirochetes (peak-to-peak wave amplitude of approximately 780 nm (17)) and the shallow
water layer (approximately 300 – 400 nm) resulted in the flat-wave lying parallel to the grid. We
compared the shape and diameter ($d_{ic}$) of the inner cell, as measured between the center of the
plasma membrane at each side of the cell, in those regions where the cell was curved (bend region), to those that were more linear (interbend region). Our rationale is that bending of the cell could cause compression and distortion. Because the total cell diameter varied in the regions where the periplasmic flagella reside (see below), $d_{ic}$ was used to test for compression. We found that there was no obvious distortion of the inner cell; all were circular. In addition, the $d_{ic}$ was $285 \pm 49$ nm within the bends ($n = 9$ bends on 7 cells), and $294 \pm 35$ nm in the interbend regions ($n = 7$ regions on 7 cells). These results suggest that bending of the cells did not dramatically alter the inner diameter of the cell.

The general shape of the cells was similar in many respects to previous determinations using light microscopy, standard transmission electron microscopy, and high-voltage electron microscopy (17,18). All cells appeared intact with an attached outer membrane, plasma membrane, periplasmic space, and the periplasmic flagella were clearly evident (Figures 1, 2a, Supplemental movie 1). Cells were circular in cross section with a diameter of approximately 310 nm in regions without periplasmic flagella, which compares reasonably well with results determined using high-voltage electron microscopy (330 nm) (17). At the ends of the cells, we often saw a bulb-like formation referred to as a bleb (Figure 3). These blebs did not contain periplasmic flagella, and had contents that appeared slightly denser than the cytoplasm. In addition, these structures were separated from the rest of the cell body and were continuous with the outer membrane.

Outer membrane and periplasmic space. The outer membrane was noticeably different than that seen in chemically fixed and stained cells. While the outer membrane sometimes appears as
having an irregular ruffled or uneven appearance in standard sections of fixed cells (Figure 3, reference (17); Figure 4, reference (30)), it was quite smooth in appearance in the tomograms (Figure 1, 2a). The width of the periplasmic space was significantly greater in regions containing periplasmic flagella than in regions where there were no periplasmic flagella (Figures 1, 2a, 2b, Supplemental movies 1 and 2). As measured between the centers of the outer membrane and plasma membrane, the width of the periplasmic space was 22.7 ± 3.9 nm in regions without periplasmic flagella (n = 17 spaces on 8 cells) and 49.0 ± 6.9 nm in regions containing periplasmic flagella (n = 11 spaces on 8 cells). The periplasmic space as measured between the inner and outer membranes was 16.0 ± 3.7 nm (n = 17 spaces on 8 cells) without the periplasmic flagella, and 42.3 ± 6.8 nm (11 spaces on 8 cells) in regions with the flagella.

Arrangement of basal bodies. The basal bodies of the periplasmic flagella were evident only at the ends of the cells in a subterminal region (Figure 4). The outer surface of the basal bodies, with the hook region exposed, were approximately 38 nm in diameter, which is similar to those reported for *T. primitia* (35 nm) but somewhat greater than those of *T. denticola* (29 nm) (20,32). Each basal body appeared as a ring, with a dense region in the center. Flagellar filaments could be seen emanating from the rings, and were seen to curve toward the center of the cell. The arrangement of basal bodies near the ends of the cell body was similar to that seen in negatively stained preparations as forming a somewhat linear arrangement parallel to the long axis of the cell (2,6,19), and were found to lie along a line in the terminal bending region of the cell. The spacing between the four adjacent basal bodies as seen in Figure 4 was approximately 90.8 ± 4.2 nm. In most tomograms, the peptidoglycan layer was not evident, but occasionally this putative wall layer was seen within the periplasmic space (Figure 4).
Arrangement of the periplasmic flagella. The periplasmic flagella were clearly visible in the tomograms, and their appearance was quite different as compared to their structure seen in fixed cells. These organelles formed an elegantly constructed ribbon (Figure 1,2b,5a,b, Supplemental movies 1, 2,3). The width of the ribbon was directly dependent on the number of periplasmic flagella and ranged from 92.9 nm to 160.9 nm with a mean of 120.0 ± 26.7 nm (Figure 6). The contribution of each periplasmic flagellum and surrounding region to the width of the ribbon was a mean of 19.2 ± 1.7 nm (range of 16.7-20.6 nm). Because previous results of unsheathed B. burgdorferi periplasmic flagella indicated a diameter approximately 16 nm (19), evidently there is little space (3.0 nm or less) between neighboring periplasmic flagella within the ribbon. Significantly, the flat ribbon formation by the periplasmic flagella is markedly different from that of stained and fixed cells. Previous results indicated that these structures formed a round bundle of 67 nm in diameter, and in thin sections as irregularly arranged filaments (17,30). We found that an occasional filament was separated from the ribbon and was located on the opposite side of the cell cylinder (Figure 2). In one ribbon of 158.27 nm that was composed of 9 periplasmic flagella, one of the flagellar filaments in the middle region appeared to terminate. The space that it would occupy is evident in the tomogram; the remaining flagellar filaments moved to fill in the vacated space (Figure 7). Specifically, the adjoining filaments are 19.0 nm apart at the point where the filament first disappears, and 7.4 nm apart at a distance of 141 nm. These results suggest that there are forces acting on the filaments to bring the adjoining filaments together. In one case, we observed a wide ribbon close to the plasma membrane, and another narrower ribbon with fewer filaments in one region close to the outer membrane (not shown). In all preparations, the ribbon wrapped around the body axis in a right-handed sense (Figure 5a,b, Supplemental
movies 1,2,3), which is in agreement with the high-voltage electron microscopy analysis (17). In addition, the angle subtended by the ribbon was approximately 1.13 radians in the interbend regions (Figure 5a), which is also in agreement with the high-voltage electron microscopy analyses (17).

**DISCUSSION**

Electron cryotomography revealed that *B. burgdorferi* produced outer membrane blebs, and these structures were located at the end of the cells. These blebs contained electron dense material. *B. burgdorferi* is known to spontaneously generate membrane vesicles when grown *in vitro* and *in vivo* (11,16). These vesicles, which are purified by passing culture supernatants though a 2 µm filter, are less than 0.25 µm in diameter and have been shown to contain DNA and several outer membrane associated proteins, such as OspA and OspB, and an 83 kDa protein (11,16,42). In many species of bacteria, vesicles are postulated to play important roles in protein secretion, virulence, and promoting cell-cell communication in biofilms (4,26,41). In *B. burgdorferi*, vesicles are believed to be able to readily penetrate tissues and initiate inflammation (42). Little is known about the precise details of how these structures are formed in other bacteria, but they are associated with the formation of blebs on the bacterial surface (26). In *B. burgdorferi*, we found that the blebs containing dense material are an extension of the outer membrane, which is in agreement with previous results of others using transmission electron microscopy (16). We find, however, that these blebs are primarily located at the ends of cells, and not along the length of the cell as previously described (16).
The width of the periplasmic space in the region where no periplasmic flagella reside is consistent with what is found for *T. denticola*, but thinner than in other gram negative bacteria.

To compare our results with published values, we made two measurements. If we measured this space between the region of highest density of both the outer membrane and inner membrane, it was 22.7 nm. This is the easier measurement to make, because the region of the membrane of highest density is quite clear. On the other hand, if we just measured the space between the inner and outer membrane, it was considerably less, 16.0 nm. However, in this case, it is more difficult to determine the boundaries of the membrane, as some clarity is lost. When measuring between the outer and inner membranes, electron microscopy of ultramicrotome sections of frozen-hydrated cells revealed a periplasmic space of 24 nm for *P. aeruginosa* and 22 nm for *E. coli* (27), but as with our results, there was considerable variability. In *T. denticola*, the periplasmic space between the membranes was reported to be 15 to 22 nm (20), which is similar to our results with *B. burgdorferi*. For *T. primitia*, the periplasmic space was found to be 28 nm, as measured by the line of highest density between the membranes, which is somewhat wider than for *B. burgdorferi*. In *T. primitia*, two layers of wall are believed to be present in the periplasmic space (33), which is different than that reported for *B. burgdorferi*.

The periplasmic space in *B. burgdorferi* contains the peptidoglycan layer, but we were not able to consistently visualize this structure, apparently due to its generally low density in this organism. Nevertheless, we know that peptidoglycan is present in these bacteria from chemical analyses, susceptibility to lysozyme after outer membrane disruption, and sensitivity to specific peptidoglycan wall acting antibiotics (3,38,39,45). Recent experiments suggest that the peptidoglycan of *B. burgdorferi* is quite flexible compared to sacculi from *E. coli* (C.
Dombrowski, W. Kan, M. A. Motaleb, N.W. Charon, R. E. Goldstein, and C. W. Wolgemuth, submitted for publication), which may be related to a decrease in peptidoglycan crosslinking compared to other bacteria. In contrast to *B. burgdorferi*, both *T. primitia* and *T. denticola* have relatively rigid helical cell cylinders, and in both cases the peptidoglycan can be more readily visualized (20,33,37).

The periplasmic space in the vicinity where the periplasmic flagella reside was notably wider (42.3 nm) than in the region without these organelles (16.0 nm). Similar results of having an increase width in the periplasmic space where the periplasmic flagella reside have been noted for *T. primitia* and *T. denticola* (20,33). In *T. denticola*, the increase in width in this region was less than the diameter of the periplasmic flagella, and the suggestion was made that the peptidoglycan layer was thinner in that region. (20,33). In contrast, in *B. burgdorferi*, this space is greater than the diameter of the periplasmic flagella (16 nm, (19). Therefore, our results suggest that the outer membrane fits tightly about the cell cylinder and that the periplasmic space widens due to the obstruction produced by the periplasmic flagella. In addition, in the bend regions of the cell, the periplasmic flagella may pull in toward the cell axis, causing the periplasmic space to widen more than the diameter of the filaments (see for example Figure 3 of reference (17)).

In contrast to plastic embedded cells, which indicated that the periplasmic flagella form a bundle in the periplasmic space (30), electron cryotomography revealed that the periplasmic flagella from a tight ribbon in this space as they wrap around the cell cylinder. The periplasmic flagella are reported to have a diameter of 16 nm (unsheathed) and 21 nm (sheathed) (19). The sheath is associated with FlaA, and it is localized proximal to the hook region (S. Shabata, S. Aizawa, M.
Motaleb, N. Charon, unpublished). Given a diameter of 16 nm per filament, we estimate that there is approximately 3 nm between each filament in the ribbon. In addition, in agreement with the high-voltage electron microscopy of imbedded cells (17), the ribbon was found to wrap around the body axis in a right handed sense. These results indicate that each periplasmic flagellum is tightly associated with the cell cylinder, and thus each can equally participate in dictating the flat wave morphology. One obvious question is the following: How does the tight ribbon of periplasmic flagella form? Perhaps the spacing of the insertion of the periplasmic flagella is critical for ribbon formation, as these organelles are inserted on a line along the subterminal ends of the cell (2,6,19), which we find is approximately 90 nm apart. In addition, formation of the ribbon may depend on having active periplasmic flagella that rotate in a manner similar to bundle formation of *E. coli* and *S. enterica* (43). Previous results indicate that *B. burgdorferi* cells incubated in the cold, or cells treated with the ionophore carbonyl cyanide-m-chlorophenylhydrazone (CCCP) that dissipates the proton gradient, become immotile, but still retain the flat wave morphology (18,30). One possible explanation for these results is that cells treated in this manner could have already formed the ribbon structure before treatment. Thus, flagellar rotation may be necessary to form but not maintain the ribbon structure. We observed that if a filament is terminated before the others in a ribbon, the adjoining periplasmic flagella move to occupy the space. These results suggest that a force brings the periplasmic flagella together in a ribbon, and it may be that flagellar rotation helps produce this force. We expect that future experiments targeting genes that result in mutants that retain their periplasmic flagella but result in paralysis will allow us to determine the role of flagellar rotation and ribbon formation. Several questions remain unanswered. Does the ribbon configuration minimize interference between flagella as they rotate? In Figure 2, one of the periplasmic flagella is separated from the
ribbon. Is this separation a consequence of that filament rotating in the opposite direction relative
to the others? In addition, we observed in one ribbon there was a region where there were two
layers of ribbons. It is not known if the periplasmic flagella from one end forms the double layer,
or if one layer is derived from the filaments from the other end. Finally, if most of the
periplasmic flagella interact with the peptidoglycan layer as in a ribbon, how does an individual
filament impact cell shape? Perhaps mutant analysis would allow the determination of how cell
shape is influenced by the number of periplasmic flagella.

A final interesting question is: What role does this ribbon structure play in the motility of *B.
burgdorferi*? We assume that the shape of the cell is explicitly tied to motility; i.e., optimal
swimming is dependent on the flat-wave shape. Theoretical work on the morphology of *B.
burgdorferi* suggests that the flat-wave shape depends strongly on the stiffness of the flagella
compared to that of the cell cylinder (C. Dombrowski, W. Kan, M. A. Motaleb, N.W. Charon, R.
E. Goldstein, and C. W. Wolgemuth, submitted for publication). If this model is correct, then
there is a minimum number of flagella required to produce a flat-wave shape with a reasonable
amplitude. For more than one flagellum attached at each end, the flagella need to be localized to
a small region about the circumference of the cell. If motility requires that the rotation of the
flagella exert force directly on the cell cylinder to generate the backward-moving flat-waves,
then a ribbon is clearly beneficial. In a bundle configuration, some of the flagella would not be in
contact with the cell cylinder and would therefore be exerting force on the other flagella, whereas
in a ribbon, each flagellum is able to exert force directly on the cell cylinder. We expect that a
detailed understanding of both ribbon formation and its physical interaction with the cell cylinder
will lead to a more complete mechanical model of how flagellar rotation and wave deformations drive motility.
FIGURES

Figure 1. Electron cryotomography cross-section, 1.8 nm thick. The outer membrane (OM), plasma membrane (PM), periplasmic flagella (PFs), and the periplasmic space (PS) are identified. Note the circular shape of the plasma membrane, the ribbon formation of the periplasmic flagella, and that the periplasmic space is wider in the domain where the periplasmic flagella reside. Bar = 50 nm.

Figure 2a. 1.8-nm-thick longitudinal slice of cell body. The outer membrane (OM), plasma membrane (PM), periplasmic flagella (PFs), and the periplasmic space (PS) are identified. Bar = 200 nm.

Figure 2b. 3-D view of sections of a cell showing that the periplasmic space (arrows) widens in the domain where the periplasmic flagella (red) reside. Blue represents plasma membrane and brown represents outer membrane.

Figure 3. 1.8-nm-thick longitudinal slice of cell end. The ends of the cell were often bulb-shaped. Bar = 100 nm.

Figure 4. 9-nm-thick longitudinal slice of cell end showing four basal bodies in an approximately linear arrangement (arrowheads). The putative peptidoglycan layer is indicated by the arrow. Bar = 200 nm in main image; bar = 100 nm in inset.
Figure 5a. 1.8-nm-thick longitudinal slice of a ribbon of nine periplasmic flagella. The ribbon wraps around the cytoplasmic cylinder in a right-handed sense (bottom of cell as viewed from the top). Bar = 200 nm.

Figure 5b. 3-D view of sections of a cell showing that the periplasmic flagella (red) forms a ribbon and wraps around the cell cylinder (blue) in a right-handed sense. The outer membrane is not shown.

Figure 6. Ribbon formation. The width of the ribbons as a function of the number of periplasmic flagella. The coefficient of correlation was 0.90.

Figure 7. 1.8-nm-thick longitudinal slice of a ribbon of nine periplasmic flagella. In this case, one of the flagella terminates (arrow) within the ribbon. Bar = 200 nm.
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