Supplementary Material

Spirochetes flagellar collar proteins have astounding effects in orientation of periplasmic flagella, bacterial shape, motility, and stability of motors in *Borrelia burgdorferi*

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Running title: FlbB is crucial for flagellar orientation and collar assembly

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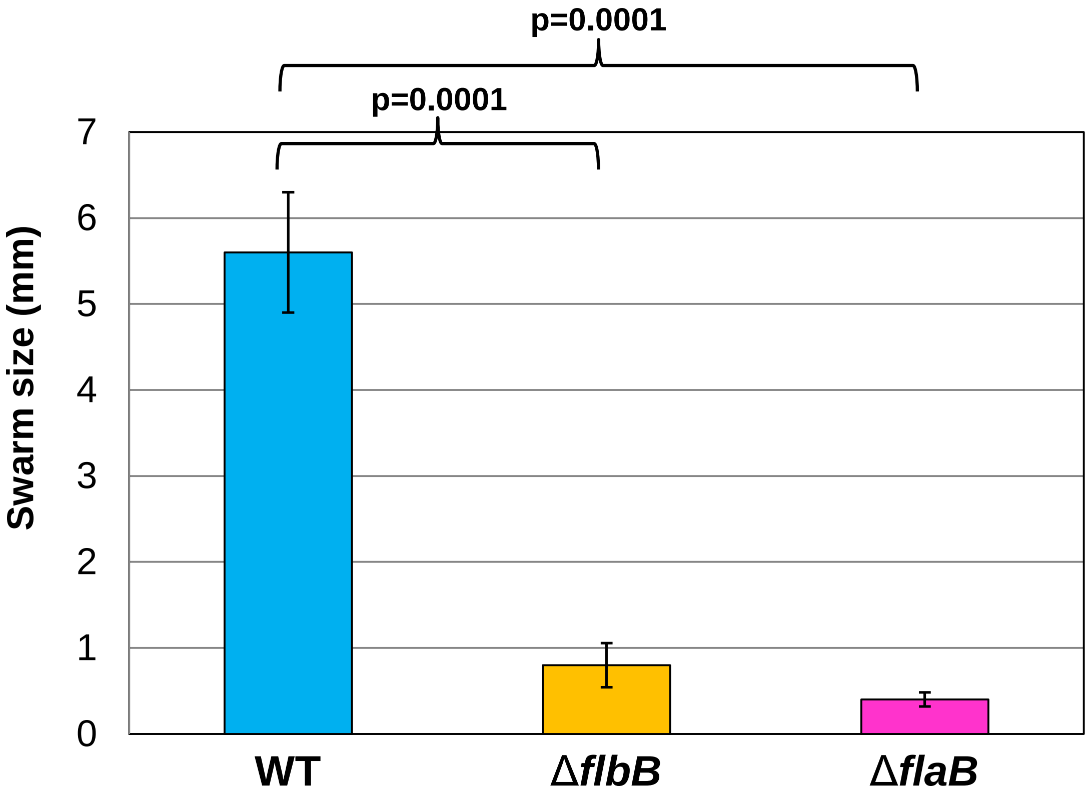


Fig S1. Δ*flbB* mutant cells are completely non-motile*.* Swarm plate motility assays in 0.4% agarose. Δ*flbB* mutant cells display significantly reduced swarming compared to WT cells. Individual colony's swarm ability was measured four weeks after inoculation. The colony size of each clone is measured in millimeters. Non-motile, flagellin-deficient Δ*flaB* mutant was used as a control. Values are indicative of the mean ± standard deviation of the averages from at least 3 plates (or 20 individual colonies) per strain. P-values between samples are shown at the top.

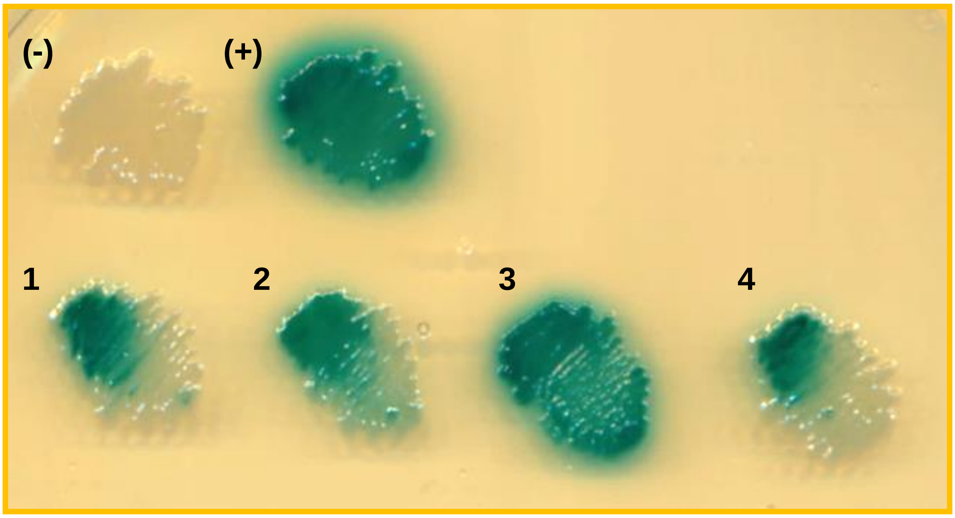
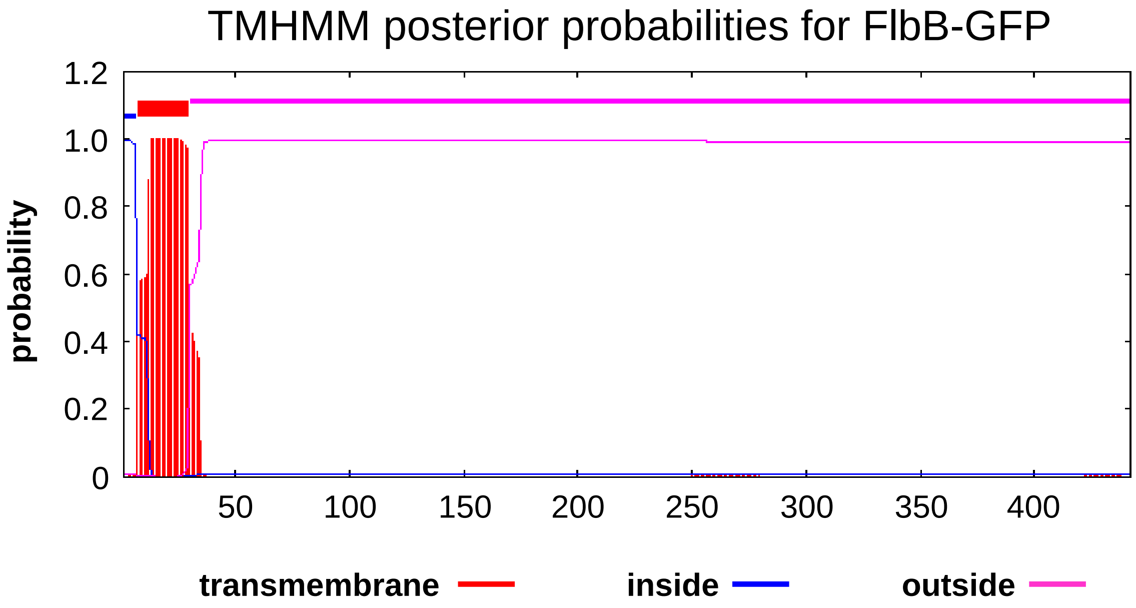


Fig S2. BACTH assays showing the interaction between FlbB and FliL, and self-interactions of FlbB-FlbB or FliL-FliL. *E. coli* cells harboring *flbB* or *fliL* gene in the vector pUT18C or pKT25 was transformed on a plate containing X-gal. Appearance of blue colored colonies indicate a positive protein-protein interaction. (**-**), negative control; (+), positive control; 1, pUT18C::*flbB*—pKT25::*fliL* co-transformant; 2, pUT18C::*fliL*—pKT25::*flbB* co-transformant; 3, pUT18C::*fliL*—pKT25::*fliL* co-transformant; 4, pUT18C::*flbB*—pKT25::*flbB* co-transformant.



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# FlbB-GFP full-length: 442 a.a. (FlbB 1-205/GFP 206-442)
# FlbB-GFP No. of predicted transmembrane (TM) helix: 1
    FlbB-GFP TMHMM2.0 inside       1-6 a.a.
    FlbB-GFP TMHMM2.0 TM helix    7-29 a.a.
    FlbB-GFP TMHMM2.0 outside     30-442 a.a.
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Fig S3. Transmembrane domain prediction of FlbB-GFP fusion protein. FlbB possesses a transmembrane domain as predicted by TMHMM Server ver. 2.0 software with default options. Predicted transmembrane region is indicated by red box (7-29 amino acid residues). FlbB-GFP amino acid sequence was used for the prediction.