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The transmembrane domains of the bacterial cell division proteins FtsB and FtsL form a stable high-order oligomer

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Abstract

FtsB and FtsL are two essential integral membrane proteins of the bacterial division complex or “divisome”, both characterized by a single transmembrane helix and a juxta-membrane coiled coil domain. The two domains are important for the association of FtsB and FtsL, a key event for their recruitment to the divisome, that in turn enables recruitment of the late divisomal components to the Z-ring and subsequent completion of the division process. Here we present a biophysical analysis performed *in vitro* that shows that the transmembrane domains of FtsB and FtsL associate strongly in isolation. Using FRET, we have measured the oligomerization of fluorophore-labeled transmembrane domains of FtsB and FtsL in both detergent and lipid. The data indicates that the transmembrane helices are likely a major contributor to the stability of the FtsB-FtsL complex. Our analyses show that FtsB and FtsL form a 1:1 higher-order oligomeric complex, possibly a tetramer. This finding suggests that the FtsB-FtsL complex is capable of multi-valent binding to FtsQ and other divisome components, a hypothesis that is consistent with the possibility that the FtsB-FtsL complex has a structural role in the stabilization of the Z-ring.

Introduction

In *Escherichia coli*, the multiprotein cell-division mediating complex, or “divisome”, comprises at least ten essential proteins, most of which are integral membrane proteins. The proteins are recruited at mid-cell over a scaffold formed by the polymeric FtsZ (the Z-ring)^{1–3}, where they assemble according to a defined stepwise pathway⁴. Two such essential components, FtsB and FtsL, occupy a place mid-way into this hierarchy^{5,6}; they are preceded by the early components (FtsZ, FtsA, ZipA, FtsK and FtsQ); in turn, they are required for the recruitment of the late divisome components (FtsW, FtsI, FtsN), which are important for the reconstruction of the cell wall.

The specific role of FtsB and FtsL is still not well understood. It has been suggested that they have a structural role in the assembly of the divisome^{6,7} and that they are important for the stabilization of the Z-ring⁸. It is clear, however, that FtsB and FtsL form a stable sub-

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Supporting Information

Supplementary Methods covering N-terminal labeling of peptides and quantification. Characterization of FITC in TFE (Figure S1). Fluorescence spectra of coumarin (donor) and FITC (acceptor) labeled peptides (Figure S2). Effect of unlabeled control peptides on FtsB homo-FRET (Figure S3). Supporting materials may be accessed free of charge online at <http://pubs.acs.org>.

complex *in vivo* prior to their association with the divisome^{9–12} and that their mutual interaction is central to their role in bacterial division.

The recruitment of FtsB and FtsL depends on their interaction with FtsQ. However, even when FtsQ is depleted these two proteins still associate with each other, and as long as they are artificially targeted to the division septum, they are still able to recruit the downstream proteins⁶. Evidence for the formation of a stable FtsB-FtsL complex is also provided by the fact that their *B. subtilis* homologs form a complex when co-expressed in *E. coli*, even though they are unlikely to interact with the significantly divergent division proteins of the host¹³. Moreover, FtsB and FtsL are quickly depleted from the cell when the proteins are not co-expressed, suggesting that they are functionally dependent on each other^{9,10,14–16}.

Determining the structural organization of the FtsB-FtsL complex, its stoichiometry, and its stability, is critical to understanding its role in division. Topologically, FtsB and FtsL are similar to each other, being small single-pass integral membrane proteins consisting of an N-terminal transmembrane (TM) helix, a juxta-membrane periplasmic coiled coil and a short C-terminal domain (Figure 1a). Previous studies indicate that both the transmembrane and the coiled coil domains are involved in their mutual association^{6,7,17}. To investigate this we recently studied the structural organization of the individual domains of FtsB in isolation¹⁸. Using an *in vivo* interaction assay, we showed that the TM domain of FtsB self-associates in *E. coli* membranes. We performed extensive mutagenesis and computed a structural model of FtsB-TM domain that is in excellent agreement with the experimental data, showing a left-handed homo-dimer mediated by an inter-helical hydrogen bond (Figure 1b). We also solved the crystal structure of the coiled coil domain of FtsB in homo-dimeric form, solubilized as a fusion construct with the viral coiled coil protein Gp7. From this evidence, we hypothesized that the FtsB-TM homodimer forms a core for the lateral association of FtsL-TM domain (Figure 1c).

Here we present a Förster Resonance Energy Transfer (FRET) based study performed *in vitro* that shows for the first time that FtsB and FtsL TM domains interact and provides new insight into the structural organization of the FtsB-FtsL complex. The data shows that the TM region of the FtsB-FtsL complex is stably folded and that the TM helices are likely a major determinant for the association of the complex. We also show that FtsL and FtsB forms a 1:1 higher-order oligomeric complex, which is consistent with our previous hypothesis that the FtsB-FtsL complex is likely a tetramer¹⁸ (Figure 1c). From these results, we hypothesize that the FtsB-FtsL complex may form a multi-valent binding hub that is important for the stabilization of the Z-ring.

Materials and Methods

Peptide sequences

The predicted TM regions of *E. coli* FtsB and FtsL (underlined sequence) were synthesized flanked by Gly amino acids on the N terminus to provide a linker between the peptide and the N-terminal fluorophore. Lys residues were added on the C terminus to improve the solubility of the peptide in aqueous media to facilitate purification of the hydrophobic TM

peptides^{19–21}. A Trp residue (in bold) was introduced in place of a native His residue in the C-terminus of FtsL-TM domain to facilitate absorbance monitoring at 280nm.

FtsB-TM: GGKLTLLLLAILVWLQYSLWFGKKKK

FtsL-TM: GGKLPCLCFICILTAVTVVTT A **WH**KK

Fmoc Solid Phase Peptide synthesis

Peptides were synthesized at 25 µmole scale on a Protein Technologies Symphony peptide synthesizer, over a low substitution (0.16mmol/g) Fmoc amide resin (Applied Biosystems) using DMF (dimethylformamide) as the solvent, 20% Piperidine with 2% DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) for deprotection and HATU (*O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate) for activation (0.40 M in DMF). Unlabeled peptides were N-terminally acetylated using standard procedures. Side chain deprotection and final cleavage from the resin was achieved using a mixture of 95:2.5:2.5 v/v of TFA (trifluoroacetic acid)/EDT(1,2-ethanedithiol)/water at room temperature for 4 hours. The cleaved peptide was precipitated using 1:10 v/v of cleavage mix:cold MTBE (methyl tertiary butyl ether) and dried in a vacuum dessicator.

On-resin N-terminal labeling of peptides

Labeling of the peptides was carried out on the peptide-resin prior to cleavage using variations of a protocol for labeling hydrophobic peptides²². Briefly, excess of a linker, N-ε-Fmoc-amino hexanoic acid (Fmoc-ε-Ahx-OH, AnaSpec) was coupled to the peptide-resin using standard coupling procedures at room temperature overnight. Following thorough washing and deprotection of the Ahx labeled peptide-resin, FITC (Fluorescein isothiocyanate) 'isomer 1' (Sigma Aldrich) was coupled with repeated washing and recoupling for ~4 days to achieve efficient labeling. For FtsL, successful coupling was obtained for the 5-FAM (5-carboxyfluorescein) version of the fluorophore in the same manner. Coupling of 7-hydroxycoumarin-3-carboxylic acid (Anaspec) was performed in a similar fashion without prior coupling of the Ahx linker. A detailed labeling procedure is provided in the Supplementary Information.

Purification of peptides

The peptides were purified on a reversed phase HPLC system (Varian ProStar 335) with a Zorbax SB-CN semi-preparative column, 5µm, 9.4 × 250mm (Agilent) for FtsB and a VP 250/10 NUCLEOSIL 100-7 C2 semi-preparative column (Macherey-Nagel) for FtsL, using a linear gradient of water and acetonitrile in the presence of 0.1% TFA. Collected fractions were lyophilized and the masses of the peptides were confirmed by a Bruker REFLEX II MALDI-TOF system using CHCA as the matrix.

Quantification of peptides

Quantification and preparation of peptide stocks in TFE (trifluoroethanol) was carried out by absorbance measurements using a Cary 50 scan UV/Vis spectrophotometer. Accurate quantification and calculation of labeling efficiencies was performed using a detailed procedure²³ described in Supplementary Information. The fluorophores were characterized

in TFE as described in Figure S1. The calculated labeling efficiencies of the purified FITC-FtsB, coumarin-FtsB, 5-FAM-FtsL and coumarin-FtsL peptides used were >95%. In spite of numerous efforts, the maximum labeling efficiency obtained for FITC-FtsL was <15%. For this reason the 5-FAM version of the fluorophore was used for the FtsL homo-FRET studies.

FRET assay

Peptides were mixed in the desired molar ratios and added to PTFE lined screw cap glass vials containing the calculated amounts of DPC (Dodecylphosphocholine) and POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) (Avanti Polar Lipids) in chloroform. The mixture was vigorously vortexed, and solvents were dried using a stream of nitrogen gas and desiccated overnight in a vacuum desiccator to remove residual organic solvents. Samples were hydrated in 10 mM HEPES, pH 7.5. Lipid samples were vortexed vigorously and equilibrated using three freeze-thaw cycles. Detergent samples were equilibrated by incubating them at room temperature for 4 hours.

For the donor+acceptor (FRET) samples, total peptide amount used was 0.5 nmoles containing 1:1 donor to acceptor molar ratios, and amount of detergent or lipid was varied to span a range of peptide:lipid/detergent molar ratios from 1:10000 to 1:300. Donor only (for 'no FRET' control) and acceptor only (for bleed-through correction) samples contained 0.25 nmoles of donor peptides and acceptor peptides, respectively, in the same range of peptide:detergent/lipid molar ratios. A sample with unlabeled FtsB:lipid ratio of 1:10000 was used as a scattering control for the lipid experiments. Triton-x 100 (0.5%) was added to coumarin-FtsB-lipid samples and fluorescence intensity measurements were taken before and after the detergent addition to account for effect of scattering on fluorescence intensity values. Fluorescence readings were taken in a HITACHI F-4500 Fluorescence Spectrophotometer. Samples were excited at 415 nm and emission scans were recorded from 425 to 650 nm at 25°C. Supplementary Figure S2 shows a characteristic FRET curve between donor and acceptor labeled FtsB peptides. FRET efficiency (E%) values were calculated using donor-quenching of coumarin emission λ_{max} at 450nm, using the formula $E = [(I_D - I_{DA})/I_D]$, where I_D is the fluorescence intensity of the donor only sample, and I_{DA} is the fluorescence intensity of the donor in the presence of the acceptor^{24,25}. Alternatively, 0.5% Triton X-100 was added to the FRET samples and incubated for 10 minutes to disrupt the liposomes and their peptide complexes, and yield a 'no FRET' control. In these cases, FRET efficiency was measured as $E = [(I_T - I_{DA})/I_T]$, where I_T is the donor emission at 450nm after Triton addition and I_{DA} is the donor emission before Triton addition²⁶. For the competition experiment (Figure 4), equimolar amounts of unlabeled FtsL were added to 1:1 donor:acceptor ratios of FtsB in a range of [total FtsB peptide]:[lipid/detergent] values of 1:10000 to 1:300. For the stoichiometric experiment (Figure 5), increasing amounts of FtsL were added to 1:1 donor:acceptor ratios of FtsB in a fixed [total FtsB peptide]:[lipid] ratio of 1:1000. Experiments were performed at least in triplica.

Results

The TM domain of FtsB homo-oligomerizes *in vitro*

Using a biological assay (TOXCAT), we previously established that FtsB-TM self-associates in *E. coli* membranes, while FtsL-TM appeared to be largely monomeric¹⁸. To confirm these observations *in vitro*, we measured FRET between coumarin/FITC or coumarin/5-FAM labeled peptides as a function of peptide concentration (Figure 2). Fluorescence measurements were taken by maintaining the concentration of the peptides constant while increasing the amount of detergent or lipid to vary the available “hydrophobic volume”, and for this reason peptide concentrations are expressed as peptide:detergent or peptide:lipid molar ratios²⁷. FRET efficiency values were calculated using donor quenching at coumarin emission maxima (450 nm).

A concentration dependent increase in FRET is observed for FtsB-TM in lipid (Figure 2b), confirming that it self-associates. Fit to a monomer-dimer equilibrium, the FRET data in lipid yield an estimated dissociation constant of 9.4×10^{-4} , corresponding to a free energy of association of approximately -4 kcal/mol. A small increase in FRET is also observable in detergent (Figure 2a), but the apparent association energy appears to be very low. Consistent with our previous TOXCAT analysis¹⁸, FtsL-TM domain appears to oligomerize very weakly in both detergent and lipid environments.

The TM domains of FtsB and FtsL associate

Having confirmed *in vitro* that the FtsB-TM homo-oligomerizes, our main interest was to verify whether the TM domains of FtsB and FtsL interact, as hypothesized. Figure 3 shows the FRET data for coumarin-FtsL (donor) and FITC-FtsB (acceptor) peptides, mixed in a 1:1 ratio, and equilibrated with decreasing amounts of DPC detergent (panel a) or POPC lipid (panel b). Increase in FRET efficiency values with increasing peptide concentration demonstrates that FtsB and FtsL TM peptides indeed associate both in detergent and in lipid. In both environments, the FtsB-FtsL heterologous FRET curves grow more rapidly than the FtsB-FtsB homo-FRET, which is displayed in Figure 3 in gray for direct visual comparison. The curves in Figure 3 are fit to a monomer-tetramer equilibrium and provide estimated K_d values of 2.3×10^{-9} in DPC and 1.3×10^{-11} in POPC.

FtsB and FtsL form a higher-order oligomer

After establishing that the TM domains of FtsB and FtsL associate, the next question was whether FtsL competes with and disrupts the FtsB homo-dimer to form an FtsB-FtsL hetero-dimer, or whether FtsL associates with the FtsB homo-dimer to form a tetramer or, potentially, another higher-order complex (scheme in Figure 4a). To investigate this question, we performed a competition experiment in which an equimolar amount of unlabeled FtsL-TM peptide was added to samples containing labeled FtsB-TM donor and acceptor pairs. The data shows that addition of FtsL led to a significant increase in the FtsB-TM FRET both in detergent micelles (Figure 4b) as well as in lipid vesicles (Figure 4c). The apparent dissociation constant of the FtsB homo-oligomer in the presence of an equimolar amount of FtsL decreases by almost two orders of magnitude from 9.4×10^{-4} to 1.3×10^{-5} , corresponding to an apparent stabilization of an FtsB dimer of 2.5 kcal/mol. Control

experiments were conducted by the addition of an equimolar amount of either an unrelated polyleucine based monomeric model TM peptide (pL-3F-dC²⁸), or an equimolar amount of unlabeled FtsB (supplementary Figure S3). The unrelated peptide did not alter the FtsB homo-FRET values. Conversely, addition of unlabeled FtsB decreased FRET, as expected.

FtsB and FtsL form a 1:1 oligomer

To further investigate the stoichiometry of the FtsB-FtsL complex, we performed a titration experiment in which unlabeled FtsL was added in increasing amounts to donor and acceptor labeled FtsB in a fixed [total FtsB peptide]:[lipid] ratio, and FtsB homo-FRET was measured. Figure 5 shows that addition of unlabeled FtsL leads to a linear increase in the FRET efficiency of FtsB homo-oligomer until the FtsL:FtsB molar ratio reaches a value of approximately 1, after which the signal flattens. The data clearly indicate that the oligomer has an equal number of FtsB and FtsL molecules, which is consistent with the hypothesized tetramer (2:2). A tetrameric FtsB-FtsL is also consistent with one of the hypothesized by Villanello et al. on the basis of a bioinformatic analysis of the soluble domains²⁹. The steep linear rise in the FRET efficiency until equal stoichiometry is reached confirms that the complex is stable at the peptide:lipid ratio in which the experiment was performed (1:1000). The FRET signal compare well with Figure 4c where the FtsB-FRET increases from ~0.4 to ~0.8 FRET efficiency upon addition of equimolar amount of unlabeled FtsL.

Discussion

An important question for understanding the FtsB-FtsL complex is how their two adjacent interaction domains – the TM helix and the juxta-membrane coiled-coil region – contribute and cooperate toward the stability of the complex. We have begun addressing this question by studying the individual domains in isolation. In our previous work we reported that the TM domain of FtsB self-associates¹⁸. We used extensive mutagenesis to identify the interaction interface and used computational modeling to interpret the experimental data. The analysis produced a complementary packed homo-dimer mediated by an inter-helical hydrogen bond (Figure 1b). While a biological role for the FtsB homo-dimer is not excluded, we hypothesized that the dimer likely represents the core of an FtsB-FtsL higher oligomer. The current results provide further evidence for this hypothesis.

We have confirmed that FtsB self-associates *in vitro* in lipid bilayers (Figure 2b). The calculated free energy of association is approximately –4 kcal/mol, a value that places FtsB as a moderately stable dimer, compared to other examples from the literature³⁰. The oligomerization of FtsB is only marginal in DPC micelles (Figure 2a): its association curve is superimposable to that of FtsL, which does not associate even in lipid. The higher stability of FtsB in lipid could be in part due to the fact that a bilayer provides an environment closer to a natural membrane³¹. We note the additional possibility that the inter-helical hydrogen bonding formed by the polar Gln 16 at the FtsB dimer interface (Figure 1b) may contribute differently to the energy of oligomerization in the two environments. Polar side chains can contribute to the interaction of TM helices^{32,33}. The contribution of a polar amino acid to association depends on the net balance between any gain of hydrogen bonding and electrostatic interactions in the bound state, and any loss of favorable interactions between

the polar groups of the side chain and water in the unassociated state³⁴. For a TM helix, however, this desolvation cost is presumably lower when, in the monomeric state, the side chain is sequestered away from water into the hydrophobic core of a lipid bilayer^{32,33}. The desolvation cost may be more significant in a detergent environment, either because of the higher propensity of water molecules to penetrate deeply into a micellar environment^{35,36}, or because of the difference in the ability of the long Gln side chain to 'snorkel' toward the surface of a micelle than in a lipid.

The main highlight of the present work is that we have obtained for the first time experimental evidence that the TM domains of FtsB and FtsL interact in isolation (Figure 3) and narrowed down the stoichiometry of the complex (Figure 5). The FRET results indicate that FtsB and FtsL associate strongly in both detergent and lipid. The calculated dissociation constants obtained by fitting the coumarin-FtsL/FITC-FtsB FRET data to a theoretical monomer-tetramer equilibrium are 2.3×10^{-9} and 1.3×10^{-11} in DPC and POPC respectively, and the corresponding ΔG° values of association are -11.7 and -14.7 kcal/mol, respectively. It is again noteworthy that the association is more stable in lipid than in detergent. The high stability of the transmembrane region of the FtsB-FtsL complex postulates that this domain has an important structural, and potentially functional role.

While it is challenging to determine the precise oligomeric state of a heterologous TM complex by FRET analysis, we provide evidence that the FtsB-FtsL complex is a higher oligomer with a 1:1 stoichiometric ratio. The observed increase of FtsB self-association in the presence of an equimolar amount of FtsL both in lipids and in detergent clearly indicates that FtsL has a strong effect in promoting the formation of a complex that contains more than one FtsB molecule (Figure 4). When the relative amount of FtsL is varied compared to FtsB, we found that the apparent FtsB stabilization increases linearly until a 1:1 FtsB-FtsL molar ratio is reached, at which point the curve sharply flattens (Figure 5).

The observed stabilization of FtsB self-association is due to a specific interaction with FtsL as opposed to non specific FRET due to crowding, as supported by two controls (Figure S3). The addition of an unrelated unlabeled monomeric model TM peptide does not perturb the FtsB self-association equilibrium, indicating that the increase in FtsB FRET is a sequence dependent effect of FtsL. On the other hand, the addition of unlabeled FtsB results in a reduction of FtsB FRET consistent with the expected competition and thus apparent dissociation of the labeled peptide pairs.

The FRET efficiency grows very rapidly as a function of concentration in the competition experiment in Figure 4, compared to the heterologous FtsB/FtsL FRET in Figure 3. It should be noted here that the concentration in Figure 3 is expressed for total peptide (FtsB+FtsL) while in Figure 4 it is expressed for FtsB peptide only, to allow direct comparison with the FtsB homo-FRET. In addition, the FtsB-FtsL experiment is only sensitive to formation of hetero-oligomers, while the competition experiment reports FRET due to FtsB homo-dimer formation as well as hetero-oligomers. It is, however, possible that other factors may also influence the analysis. For example imprecision in the difficult quantification of all labeled and unlabeled species could render the peptide molar ratios inaccurate. Moreover, the calculation of the reported dissociation constants are based on FRET efficiency, which was

obtained from donor quenching at its emission maximum. This quantity may contain contributions from self quenching of the donor as well as colocalization effects due to random proximity of donors and acceptors as peptides diffuse randomly in bilayers even in low peptide concentrations. Accounting for these effects could in principle lower the actual FRET efficiency values and yield more precise thermodynamics of association. Furthermore, proximity effects due to different orientation in the opposite bilayer leaflets of multi-lamellar vesicles could also lead to spurious increased FRET efficiency values that have variable contributions for different FRET pairs^{25,37}. Nonetheless, it is reasonable to infer that the contribution of non specific FRET due to all these factors is comparable across experiments performed in similar experimental conditions with the same FRET pair. Therefore, while from a rigorously quantitative stand point this report is an initial thermodynamic analysis, we note that, overall, the magnitude and the consistency of the data across the different experiments provide strong evidence that the TM domains of FtsB and FtsL form a stable 1:1 higher-oligomer complex, a fact that is important for understanding their biological function.

Further work is necessary to establish the precise oligomerization state of the FtsB-FtsL complex, using methods that are directly sensitive to the total mass of the complex, such as Analytical Ultracentrifugation^{38,39}. The present work, however, represents a significant step toward understanding the structural organization of the FtsB-FtsL complex and its role in the assembly and function of the divisome. It provides further evidence for our previously reported hypothesis that a tetrameric TM complex is likely formed by the lateral association of FtsL-TM helix onto a FtsB-TM dimer¹⁸, and demonstrates that the TM region of FtsB-FtsL is stable and likely a major contributor to the stability of the overall complex (Figure 1c).

The fact that FtsB and FtsL form a higher-oligomeric complex may be significant for the functional role of the proteins. FtsB and FtsL have been hypothesized to be structural proteins that contribute to the stabilization of the Z-ring⁶⁻⁸. There is indeed substantial evidence that FtsB and FtsL bind to with multiple partners: they associate with FtsQ, an interaction that is mediate by the periplasmic domains of the proteins^{6,29,40-42}; the N-terminal tail of FtsL is important for the recruitment of FtsW⁴⁰; moreover, two-hybrid assay have identified several potential interactions with other proteins^{43,44}. This leads to the hypothesis that a tetrameric and thus multi-valent FtsB-FtsL complex may simultaneously bind multiple partners and act as an interaction hub that tethers together several complexes of the divisome. This hypothetical role is schematically illustrated in Figure 6.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

TM	transmembrane
FtsB-TM	transmembrane domain of FtsB
FtsL-TM	transmembrane domain of FtsL
FRET	Förster Resonance Energy Transfer
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
DPC	dodecylphosphocholine
FITC	fluorescein isothiocyanate
5-FAM	5-carboxyfluorescein
TFE	trifluoroethanol

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Figure 1. Starting hypothesis: FtsB and FtsL form a higher-order oligomer

a) FtsB and FtsL are two integral membrane proteins of the bacterial division complex. Their topology consists of a single transmembrane domain (TM) and a juxta-membrane coiled coil domain (CC). b) In previous work we determined that the transmembrane domain of FtsB homo-oligomerizes, and obtained an experimentally-supported computational model of a homo-dimer mediated by a critical hydrogen bond. c) We hypothesized that the TM dimer of FtsB (yellow) forms a core mediated by the critical Gln16 (red circles) for the lateral association of FtsL (blue). Here, we further provide support for this hypothesis by demonstrating that the TM domains of FtsB and FtsL associate stably in isolation forming a 1:1 higher-order oligomer.

Figure 2. FtsB self-associates *in vitro* in lipid

Concentration-dependent FRET between a coumarin/FITC donor/acceptor pair, measured in (a) DPC micelles and (b) POPC multi-lamellar vesicles. The peptide concentration is expressed as a function of “hydrophobic volume” as peptide:detergent or peptide:lipid molar ratio. a) FtsB (filled squares) and FtsL (open squares) self-associate very weakly in detergent. b) The self-association of FtsL is not enhanced in lipid, while significant association is observed for FtsB. The finding confirm the self-association of FtsB previously reported using TOXCAT¹⁸. The lines correspond to fits to monomer-dimer equilibria.

Figure 3. The TM domains of FtsB and FtsL form a stable oligomeric complex

Concentration-dependent FRET (black squares) between a coumarin-FtsL/FITC-FtsB pair, measured in a) DPC micelles and b) POPC multi-lamellar vesicles. The curves represent fits to monomer-tetramer equilibria. The FtsB self-association data (Figure 2) is reported as a reference (gray, dashed lines). The data indicate that FtsB and FtsL TM domains interact in both detergent and lipid environments.

Figure 4. The TM domains of FtsB and FtsL form a higher-order oligomeric complex

a) Schematic view of a competition experiment in which FRET for a coumarin-FtsB/FITC-FtsB donor/acceptor pair (white helices, D: donor, A: acceptor) is measured in the presence of an equimolar amount of unlabeled FtsL (black helices). The concentration-dependent FRET in DPC detergent (b) and in POPC multi-lamellar vesicles (c) is reported as a function of FtsB peptide concentration (not as FtsB+FtsL total peptide) as black squares. The FtsB self-association data (Figure 2) is repeated here as a reference (gray, dashed lines). The data is consistent with an FtsL-dependent stabilization of an FtsB homo-oligomer (right-most equilibrium in panel a) rather than a competing disruption (left-most equilibrium), suggesting the formation of a higher-order FtsB-FtsL oligomer.

Figure 5. FtsB and FtsL form a 1:1 complex

Titration experiment in which unlabeled FtsL is added to donor and acceptor labeled FtsB, in a 1:1000 FtsB peptide to lipid ratio. The data shows a steep increase of FtsB homo-FRET until the stoichiometric ratio with FtsL reaches 1:1, followed by a sharp plateauing of the signal. The data is consistent with formation of a tetrameric (2:2) or hexameric (3:3) or higher complex. The lines represent two linear regressions to the set of molar ratio points from 0 to 1, and to those with ratios from 1 to 8..

Figure 6. A higher-oligomeric FtsB-FtsL complex may be a multi-valent tethering structural element of the divisome

A schematic depiction of a tetrameric FtsB-FtsL complex (black and white circles marked B and L) seen from above the plane of the membrane. The FtsB-FtsL complex is bound to FtsQ with the periplasmic tail of FtsB^{6,42} and to FtsW with the cytoplasmic tail of FtsL⁴⁰. Other hypothetical divisome components are depicted as dotted circles. The scheme illustrates how a tetrameric FtsB-FtsL complex could potentially bind to multiple divisome elements at the same time, therefore acting as a tethering structural element that contributes to holding together multiple sub-complexes within the Z-ring.