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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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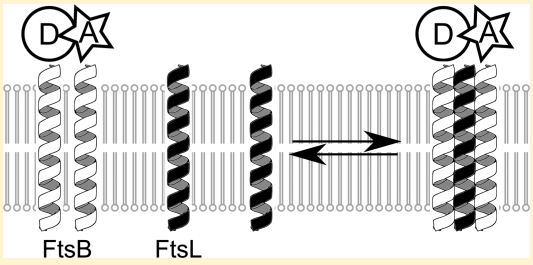
Supporting Information

**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 juxtamembrane coiled coil domain. The two domains are important for the association of FtsB and FtsL, a key event for their recruitment to the divisome, which in turn allows the 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 Förster resonance energy transfer, we have measured the oligomerization of fluorophore-labeled transmembrane domains of FtsB and FtsL in both detergent and lipid. The data indicate 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 multivalent 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.




In *Escherichia coli*, the multiprotein cell division-mediating complex, or "divisome", comprises at least 10 essential proteins, most of which are integral membrane proteins. The proteins are recruited at midcell over a scaffold formed by the polymeric FtsZ (the Z-ring),[1−3] where they assemble according to a defined stepwise pathway.[4] Two such essential components, FtsB and FtsL, occupy a place midway into this hierarchy;[5,6] they are preceded by the early components (FtsZ, FtsA, ZipA, FtsK, and FtsQ), and in turn, they are required for the recruitment of the late divisome components (FtsW, FtsI, and 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-ring.[8] It is clear, however, that FtsB and FtsL form a stable subcomplex *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 proteins.[6] Evidence of the formation of a stable FtsB−FtsL complex is also provided by the fact that their *B. subtilis* homologues form a complex when co-expressed in *E. coli*, even though they are unlikely to interact with the significantly divergent division proteins of the host.[13] 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 juxtamembrane 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 isolation.[18] 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 the FtsB TM domain that is in excellent agreement with the experimental data, showing a left-handed homodimer mediated by an interhelical hydrogen bond (Figure 1b). We also determined the crystal structure of the coiled coil domain of FtsB in homodimeric 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 the 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

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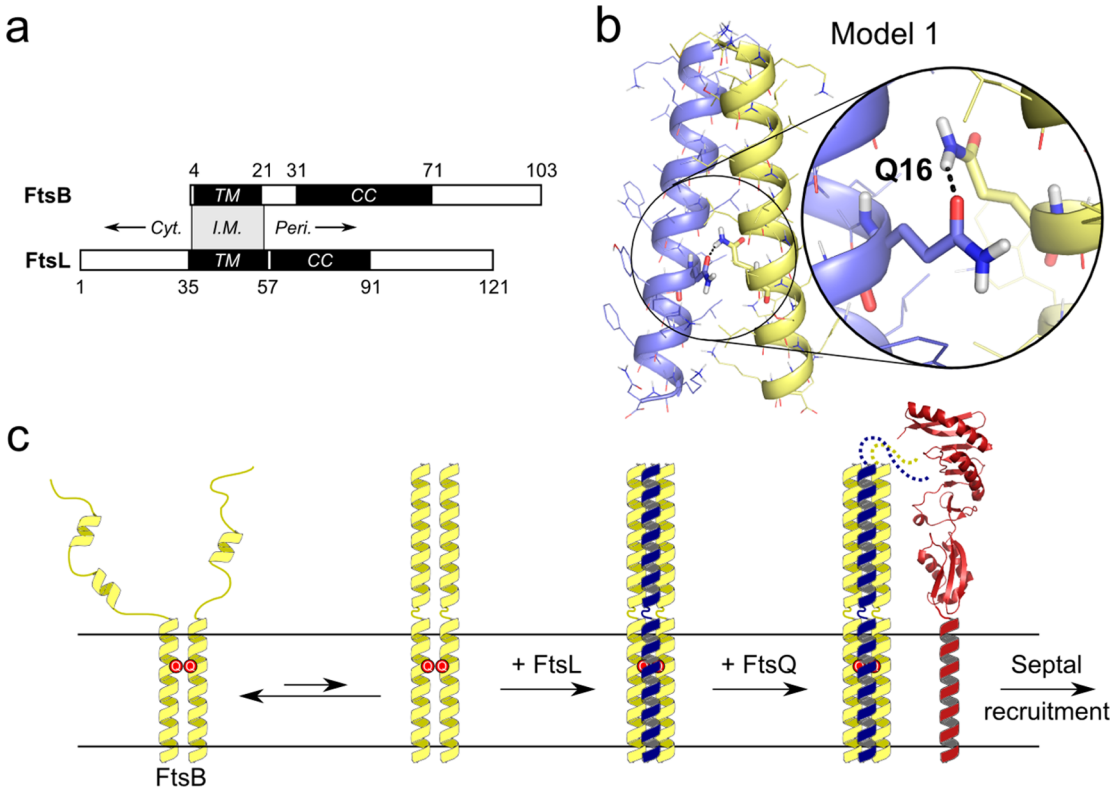


**Figure 1.** Starting hypothesis that FtsB and FtsL form a higher-order oligomer. (a) FtsB and FtsL are two integral membrane proteins of the bacterial division complex. Their topology consists of a single transmembrane (TM) domain and a juxtamembrane coiled coil (CC) domain. (b) In previous work, we determined that the transmembrane domain of FtsB homo-oligomerizes and obtained an experimentally supported computational model of a homodimer 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.

complex. The data show that the TM region of the FtsB−FtsL complex is stably folded and that the TM helices are likely a major determinant of the association of the complex. We also show that FtsL and FtsB form a 1:1 higher-order oligomeric complex, which is consistent with our previous hypothesis that the FtsB−FtsL complex is likely a tetramer[18] (Figure 1c). From these results, we hypothesize that the FtsB−FtsL complex may form a multivalent 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 at the N-terminus to provide a linker between the peptide and the N-terminal fluorophore. Lys residues were added to 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 (bold) was introduced in place of a native His residue in the C-terminus of the FtsL TM peptide to facilitate absorbance monitoring at 280 nm.

FtsB-TM: GGKLTLLLLAILVWLQYSLWFGKKKK

FtsL-TM: GGGKLPLCLFICIILTAVTVVTTA**W**HKK

**Fmoc Solid Phase Peptide Synthesis.** Peptides were synthesized on a 25 μmol scale on a Protein Technologies Symphony peptide synthesizer, over a low-substitution (0.16 mmol/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 were achieved using a 95:2.5:2.5 (v/v) mixture of TFA (trifluoroacetic acid), EDT (1,2-ethanedithiol), and water at room temperature for 4 h. The cleaved peptide was precipitated using a 1:10 (v/v) mixture of cleavage mix and cold MTBE (methyl tertiary butyl ether) and dried in a vacuum desiccator.

**On-Resin N-Terminal Labeling of Peptides.** Labeling of the peptides was conducted on the peptide-resin prior to cleavage using variations of a protocol for labeling hydrophobic peptides.[22] Briefly, an excess of a linker, Fmoc-ε-Ahx-OH (*N*-ε-Fmoc-aminohexanoic acid) (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


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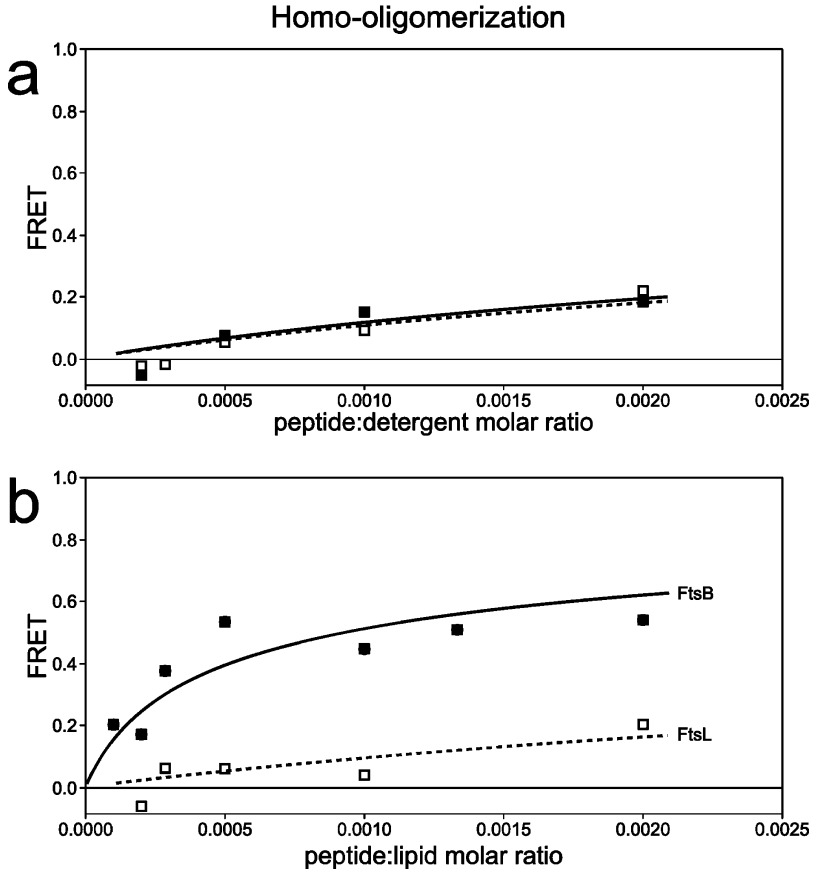


**Figure 2.** FtsB self-associates *in vitro* in lipid. Concentration-dependent FRET between a coumarin/FITC donor/acceptor pair, measured in (a) DPC micelles and (b) POPC multilamellar vesicles. The peptide concentration is expressed as a function of "hydrophobic volume" as peptide:detergent or peptide:lipid molar ratio. (a) FtsB (■) and FtsL (□) 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 confirms the self-association of FtsB previously reported using TOXCAT.[18] The lines correspond to fits to monomer−dimer equilibria.

fashion without prior coupling of the Ahx linker. A detailed labeling procedure is provided in the Supporting Information.

**Purification of Peptides.** The peptides were purified on a reversed phase high-performance liquid chromatography system (Varian ProStar 335) with a Zorbax SB-CN semi-preparative column [5 $\mu$m, 9.4 mm × 250 mm (Agilent)] for FtsB and a VP 250/10 NUCLEOSIL 100-7 C2 semipreparative 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 (alpha-cyano-4-hydroxycinnamic acid) as the matrix.

**Quantification of Peptides.** Quantification and preparation of peptide stocks in TFE (trifluoroethanol) were conducted by absorbance measurements using a Cary 50 scan UV−vis spectrophotometer. Accurate quantification and calculation of labeling efficiencies were performed using a detailed procedure[23] described in the Supporting Information. The fluorophores were characterized in TFE as described in Figure S1 of the Supporting Information. 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 maximal labeling efficiency obtained for the FITC−FtsL peptide 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 h.

For the donor−acceptor (FRET) samples, the total peptide amount used was 0.5 nmol containing a 1:1 donor:acceptor molar ratio, and the 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 nmol of donor peptides and acceptor peptides, respectively, in the same range of peptide:detergent/lipid molar ratios. A sample with an 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 addition of detergent to account for the effect of scattering on fluorescence intensity values. Fluorescence readings were taken in a HITACHI F-


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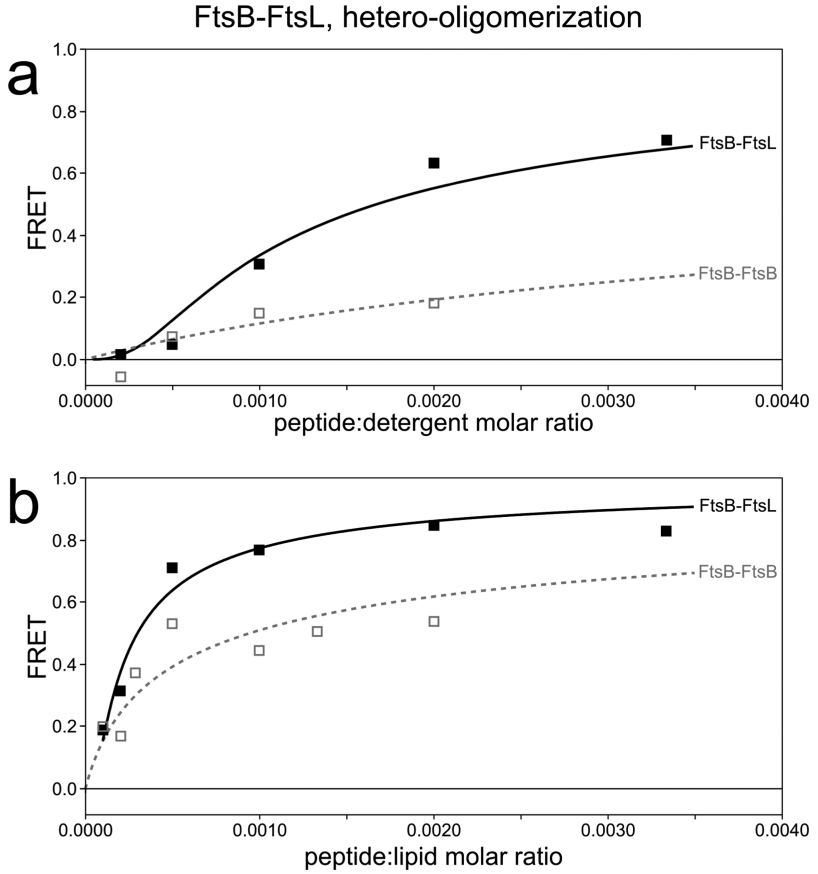


**Figure 3.** TM domains of FtsB and FtsL form a stable oligomeric complex. Concentration-dependent FRET (■) between a coumarin−FtsL/FITC−FtsB pair, measured in (a) DPC micelles and (b) POPC multilamellar vesicles. The curves represent fits to monomer−tetramer equilibria. The FtsB self-association data (Figure 2) are reported as a reference (gray, dashed lines). The data indicate that FtsB and FtsL TM domains interact in both detergent and lipid environments.

4500 fluorescence spectrophotometer. Samples were excited at 415 nm, and emission scans were recorded from 425 to 650 nm at 25 °C. Figure S2 of the Supporting Information shows a characteristic FRET curve between donor- and acceptor-labeled FtsB peptides. FRET efficiency ($E$%) values were calculated using donor quenching of coumarin emission $\lambda_{max}$ at 450 nm, 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 min to disrupt the liposomes and their peptide complexes and yielded a no FRET control. In these cases, the FRET efficiency was determined with the equation $E = [(I_T - I_{DA})/I_T]$, where $I_T$ is the donor emission at 450 nm after the addition of Triton and $I_{DA}$ is the donor emission before the addition of Triton.[26] 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 ratios 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 triplicate.

## RESULTS

**The TM Domain of FtsB Homo-Oligomerizes *in Vitro*.** Using a biological assay (TOXCAT), we previously established that the FtsB TM homodimer self-associates in *E. coli* membranes, while the FtsL TM homodimer appeared to be largely monomeric.[18] 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 ratios.[27] FRET efficiency values were calculated using donor quenching at coumarin emission maxima (450 nm).

A concentration-dependent increase in the extent of FRET is observed for the FtsB TM homodimer 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 $9.4 \times 10^{-4}$, corresponding to a free energy of association of approximately −4 kcal/mol. A small increase in the extent of FRET can also be observed in detergent (Figure 2a), but the apparent association energy appears to be very low. Consistent with our previous TOXCAT analysis,[18] the FtsL TM domain appears to homo-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)


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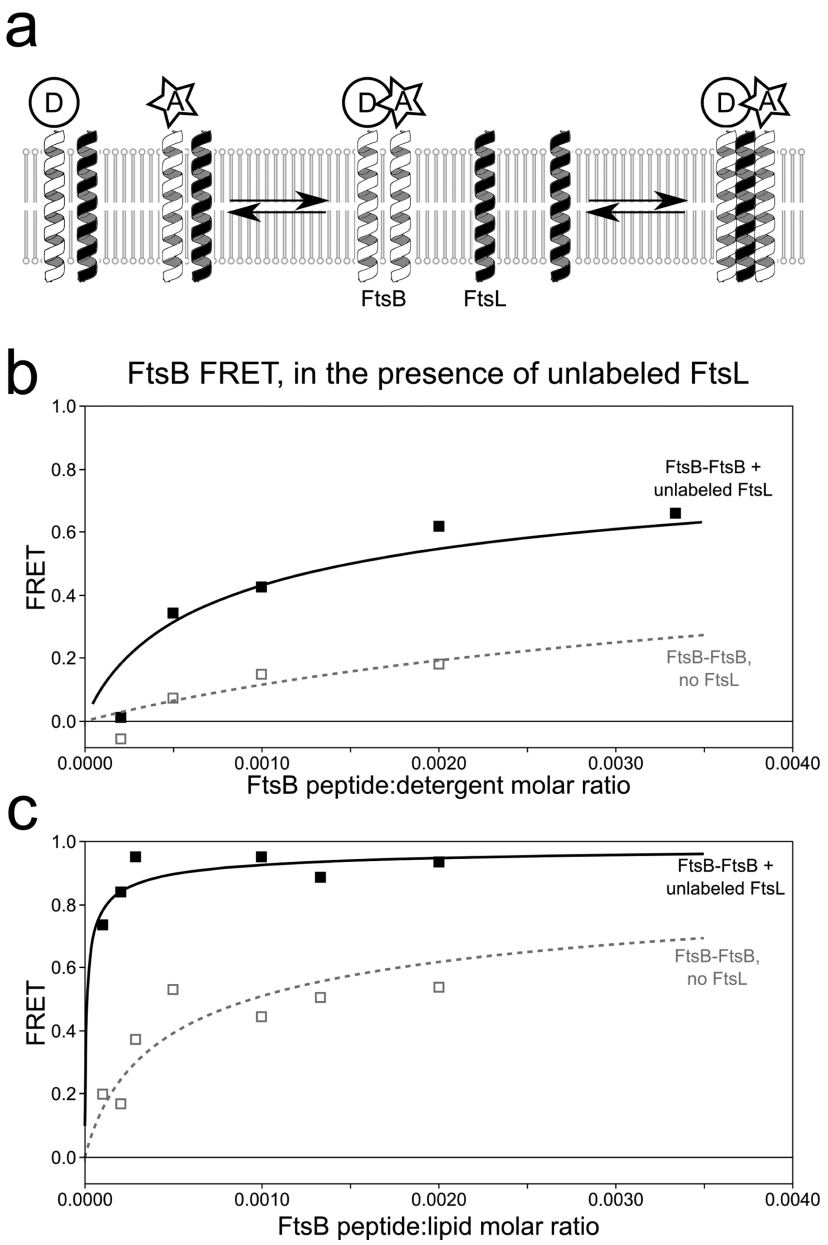


**Figure 4.** TM domains of FtsB and FtsL form a higher-order oligomeric complex. (a) Schematic view of a competition experiment in which FRET for a coumarin−FtsB/FITC−FtsB donor/acceptor pair (white helices, D for donor and A for acceptor) is measured in the presence of an equimolar amount of unlabeled FtsL (black helices). The concentration-dependent FRET (■) in DPC detergent (b) and in POPC multilamellar vesicles (c) is reported as a function of FtsB peptide concentration (not as FtsB+FtsL total peptide). The FtsB self-association data (Figure 2) are repeated here as a reference (gray, dashed lines). The data are 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.

peptides, mixed in a 1:1 ratio, and equilibrated with decreasing amounts of DPC detergent (panel a) or POPC lipid (panel b). An increase in FRET efficiency values with an 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 curves, 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 $2.3 \times 10^{-9}$ in DPC and $1.3 \times 10^{-11}$ 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 homodimer to form an FtsB−FtsL heterodimer or whether FtsL associates with the FtsB homodimer 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 show that addition of FtsL led to a significant increase in the level of FtsB-TM FRET both in detergent micelles (Figure 4b) and 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 2 orders of magnitude from $9.4 \times 10^{-4}$ to $1.3 \times 10^{-5}$, corresponding to an apparent stabilization of an FtsB dimer of 2.5 kcal/mol. Control experiments were


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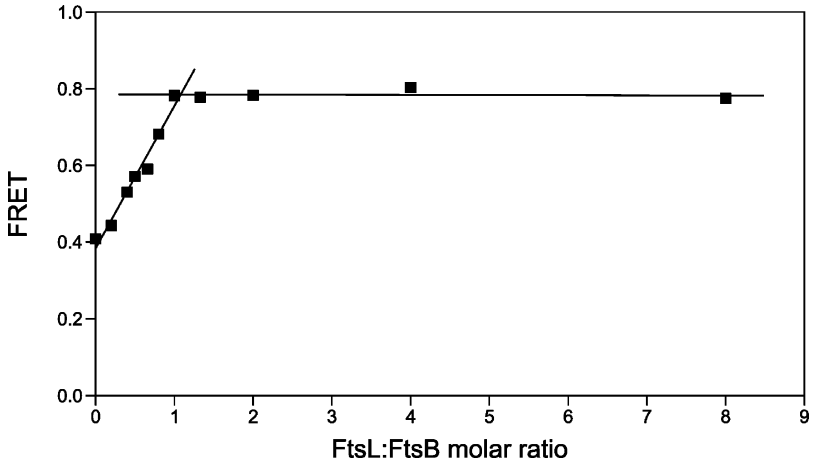


**Figure 5.** FtsB and FtsL form a 1:1 complex. Titration experiment in which unlabeled FtsL is added to donor- and acceptor-labeled FtsB, in a 1:1000 FtsB peptide:lipid ratio. The data show a steep increase in the level of FtsB homo-FRET until the stoichiometric ratio with FtsL reaches 1:1, followed by a sharp plateauing of the signal. The data are consistent with formation of a tetrameric (2:2), hexameric (3:3), or higher-order 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.

conducted by addition of an equimolar amount of either an unrelated polyleucine-based monomeric model TM peptide (pL-3F-dC[28]) or an equimolar amount of unlabeled FtsB (Figure S3 of the Supporting Information). The unrelated peptide did not alter the FtsB homo-FRET values. Conversely, addition of unlabeled FtsB decreased the level of 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 the FtsB homo-oligomer until the FtsL:FtsB molar ratio reaches a value of approximately 1, after which the signal becomes flattened. 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 complex is also consistent with one of those hypothesized by Villanello et al. on the basis of a bioinformatic analysis of the soluble domains.[29] The steep linear rise in the FRET efficiency until equal stoichiometry is reached confirms that the complex is stable at the peptide:lipid ratio at which the experiment was performed (1:1000). The FRET signal compares well with Figure 4c where the FtsB FRET increases from ∼0.4 to ∼0.8 FRET efficiency upon addition of an 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 juxtamembrane coiled coil region, contribute to and cooperate in 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-associates.[18] We used extensive mutagenesis to identify the interaction interface and used computational modeling to interpret the experimental data. The analysis produced a complementary packed homodimer mediated by an interhelical hydrogen bond (Figure 1b). While a biological role for the FtsB homodimer is not excluded, we hypothesized that the dimer likely represents the core of an FtsB−FtsL higher-order oligomer. These results provide further evidence of 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 literature.[30] The oligomerization of FtsB is only marginal in DPC micelles (Figure 2a): its association curve can be superimposed on 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 membrane.[31] We note the additional possibility that the interhelical hydrogen bonding formed by the polar Gln16 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 state.[34] For a TM helix, however, this desolvation cost is presumably lower when, in the monomeric state, the side chain is sequestered 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 stronger 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 versus that in a lipid.

The main highlight of this 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 $2.3 \times 10^{-9}$ and $1.3 \times 10^{-11}$ in DPC and POPC, respectively, and the corresponding $\Delta G^{\circ}$ 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


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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-order oligomer with a 1:1 stoichimetric ratio. The observed increase in the level of FtsB self-association in the presence of an equimolar amount of FtsL both in lipid 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 that of FtsB, we found that the level of apparent FtsB stabilization increases linearly until a 1:1 FtsB:FtsL molar ratio is reached, at which point the curve is sharply flattened (Figure 5).

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

The FRET efficiency increases very rapidly as a function of concentration in the competition experiment in Figure 4, compared to the heterologous FtsB/FtsL FRET efficiency in Figure 3. It should be noted here that the concentration in Figure 3 is expressed for the total peptide (FtsB and FtsL), while in Figure 4, it is expressed for the FtsB peptide only, to allow direct comparison with the FtsB homo-FRET. In addition, the FtsB−FtsL experiment is sensitive only to formation of hetero-oligomers, while the competition experiment reports FRET due to formation of the FtsB homodimer and 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 is 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 at 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 orientations in the opposite bilayer leaflets of multilamellar 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 nonspecific FRET due to all these factors is comparable across experiments performed under similar experimental conditions with the same FRET pair. Therefore, while from a rigorously quantitative standpoint 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-order oligomeric complex, a fact that is important for understanding their biological function.

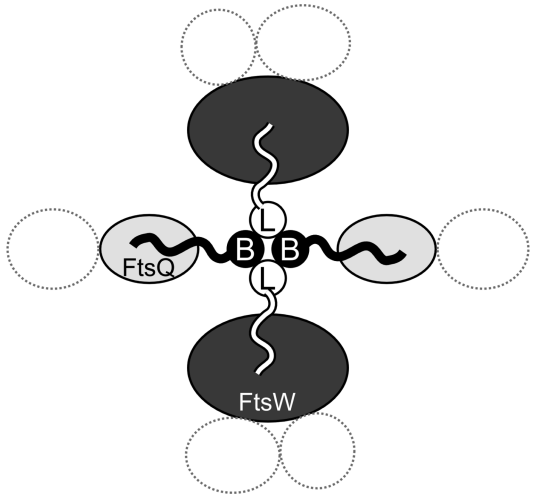


**Figure 6.** Higher-order oligomeric FtsB−FtsL complex may be a multivalent tethering structural element of the divisome. A schematic depiction of a tetrameric FtsB−FtsL complex (black and white circles marked B and L, respectively) seen from above the plane of the membrane. The FtsB−FtsL complex is bound to FtsQ with the periplasmic tail of FtsB[6,42] and to FtsW with the cytoplamic tail of FtsL.[40] 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 subcomplexes within the Z-ring.

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] This 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 the FtsL TM helix onto a FtsB TM dimer,[18] and demonstrates that the TM region of the FtsB−FtsL complex 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-order 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-ring.[6−8] There is indeed substantial evidence that FtsB and FtsL bind to multiple partners: they associate with FtsQ, an interaction that is mediated by the periplasmic domains of the proteins;[6,29,40−42] the N-terminal tail of FtsL is important for the recruitment of FtsW;[40] moreover, two-hybrid assays have identified several potential interactions with other proteins.[43,44] This leads to the hypothesis that a tetrameric and thus multivalent 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.

## ■ ASSOCIATED CONTENT

### Supporting Information

Supplementary methods covering the 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), and the effect of unlabeled control peptides on FtsB homo-FRET (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

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## ■ ABBREVIATIONS

TM, transmembrane; FtsB-TM, transmembembrane domain of FtsB; FtsL-TM, transmembembrane 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.

## ■ REFERENCES


(1) Adams, D. W., and Errington, J. (2009) Bacterial cell division: Assembly, maintenance and disassembly of the Z ring. *Nat. Rev. Microbiol. 7*, 642−653.

(2) Mingorance, J., Rivas, G., Vélez, M., Gómez-Puertas, P., and Vicente, M. (2010) Strong FtsZ is with the force: Mechanisms to constrict bacteria. *Trends Microbiol. 18*, 348−356.

(3) Erickson, H. P., Anderson, D. E., and Osawa, M. (2010) FtsZ in Bacterial Cytokinesis: Cytoskeleton and Force Generator All in One. *Microbiol. Mol. Biol. Rev. 74*, 504−528.

(4) Goehring, N. W., and Beckwith, J. (2005) Diverse Paths to Midcell: Assembly of the Bacterial Cell Division Machinery. *Curr. Biol. 15*, R514−R526.

(5) Goehring, N. W., Gonzalez, M. D., and Beckwith, J. (2006) Premature targeting of cell division proteins to midcell reveals hierarchies of protein interactions involved in divisome assembly. *Mol. Microbiol. 61*, 33−45.

(6) Gonzalez, M. D., and Beckwith, J. (2009) Divisome under construction: Distinct domains of the small membrane protein FtsB are necessary for interaction with multiple cell division proteins. *J. Bacteriol. 191*, 2815−2825.

(7) Robichon, C., Karimova, G., Beckwith, J., and Ladant, D. (2011) Role of Leucine Zipper Motifs in Association of the *Escherichia coli* Cell Division Proteins FtsL and FtsB. *J. Bacteriol. 193*, 4988−4992.

(8) Geissler, B., and Margolin, W. (2005) Evidence for functional overlap among multiple bacterial cell division proteins: Compensating for the loss of FtsK. *Mol. Microbiol. 58*, 596−612.

(9) Buddelmeijer, N., Judson, N., Boyd, D., Mekalanos, J. J., and Beckwith, J. (2002) YgbQ, a cell division protein in *Escherichia coli* and *Vibrio cholerae*, localizes in codependent fashion with FtsL to the division site. *Proc. Natl. Acad. Sci. U.S.A. 99*, 6316−6321.

(10) Daniel, R. A., and Errington, J. (2000) Intrinsic instability of the essential cell division protein FtsL of *Bacillus subtilis* and a role for DivIB protein in FtsL turnover. *Mol. Microbiol. 36*, 278−289.

(11) Ghigo, J.-M., Weiss, D. S., Chen, J. C., Yarrow, J. C., and Beckwith, J. (1999) Localization of FtsL to the *Escherichia coli* septal ring. *Mol. Microbiol. 31*, 725−737.

(12) Katis, V. L., Wake, R. G., and Harry, E. J. (2000) Septal Localization of the Membrane-Bound Division Proteins of *Bacillus subtilis* DivIB and DivIC Is Codependent Only at High Temperatures and Requires FtsZ. *J. Bacteriol. 182*, 3607−3611.

(13) Robichon, C., King, G. F., Goehring, N. W., and Beckwith, J. (2008) Artificial Septal Targeting of *Bacillus subtilis* Cell Division Proteins in *Escherichia coli*: An Interspecies Approach to the Study of Protein-Protein Interactions in Multiprotein Complexes. *J. Bacteriol. 190*, 6048−6059.

(14) Daniel, R. A., Harry, E. J., Katis, V. L., Wake, R. G., and Errington, J. (1998) Characterization of the essential cell division gene ftsL (yllD) of *Bacillus subtilis* and its role in the assembly of the division apparatus. *Mol. Microbiol. 29*, 593−604.

(15) Daniel, R. A., Noirot-Gros, M.-F., Noirot, P., and Errington, J. (2006) Multiple Interactions between the Transmembrane Division Proteins of *Bacillus subtilis* and the Role of FtsL Instability in Divisome Assembly. *J. Bacteriol. 188*, 7396−7404.

(16) Bramkamp, M., Weston, L., Daniel, R. A., and Errington, J. (2006) Regulated intramembrane proteolysis of FtsL protein and the control of cell division in *Bacillus subtilis*. *Mol. Microbiol. 62*, 580−591.

(17) Rowland, S. L., Wadsworth, K. D., Robson, S. A., Robichon, C., Beckwith, J., and King, G. F. (2010) Evidence from Artificial Septal Targeting and Site-Directed Mutagenesis that Residues in the Extracytoplasmic Domain of DivIB Mediate Its Interaction with the Divisomal Transpeptidase PBP 2B. *J. Bacteriol. 192*, 6116−6125.

(18) LaPointe, L. M., Taylor, K. C., Subramaniam, S., Khadria, A., Rayment, I., and Senes, A. (2013) Structural organization of FtsB, a transmembrane protein of the bacterial divisome. *Biochemistry 52*, 2574−2585.

(19) Rath, A., and Deber, C. M. (2013) Design of transmembrane peptides: Coping with sticky situations. *Methods Mol. Biol. (N.Y., NY, U.S.) 1063*, 197−210.

(20) Liu, L.-P., and Deber, C. M. (1998) Guidelines for membrane protein engineering derived from de novo designed model peptides. *Pept. Sci. 47*, 41−62.

(21) Melnyk, R. A., Partridge, A. W., and Deber, C. M. (2001) Retention of Native-like Oligomerization States in Transmembrane Segment Peptides: Application to the *Escherichia coli* Aspartate Receptor. *Biochemistry 40*, 11106−11113.

(22) Stahl, P. J., Cruz, J. C., Li, Y., Michael, Yu. S., and Hristova, K. (2012) On-the-resin N-terminal modification of long synthetic peptides. *Anal. Biochem. 424*, 137−139.

(23) Khadria, A., and Senes, A. (2013) Measurement of Transmembrane Peptide Interactions in Liposomes Using Förster Resonance Energy Transfer (FRET). *Methods Mol. Biol. (N.Y., NY, U.S.) 1063*, 19−36.

(24) Li, E., You, M., and Hristova, K. (2005) Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis and Förster Resonance Energy Transfer Suggest Weak Interactions between Fibroblast

Growth Factor Receptor 3 (FGFR3) Transmembrane Domains in the Absence of Extracellular Domains and Ligands. *Biochemistry 44*, 352−360.
(25) You, M., Li, E., Wimley, W. C., and Hristova, K. (2005) Förster resonance energy transfer in liposomes: Measurements of transmembrane helix dimerization in the native bilayer environment. *Anal. Biochem. 340*, 154−164.
(26) Schick, S., Chen, L., Li, E., Lin, J., Köper, I., and Hristova, K. (2010) Assembly of the M2 Tetramer Is Strongly Modulated by Lipid Chain Length. *Biophys. J. 99*, 1810−1817.
(27) Fleming, K. G., Ren, C.-C., Doura, A. K., Eisley, M. E., Kobus, F. J., and Stanley, A. M. (2004) Thermodynamics of glycophorin A transmembrane helix dimerization in C14 betaine micelles. *Biophys. Chem. 108*, 43−49.
(28) Pace, C. J., Huang, Q., Wang, F., Palla, K. S., Fuller, A. A., and Gao, J. (2011) A FlAsH−Tetracysteine Assay for Quantifying the Association and Orientation of Transmembrane α-Helices. *ChemBioChem 12*, 1018−1022.
(29) Villanelo, F., Ordenes, A., Brunet, J., Lagos, R., and Monasterio, O. (2011) A model for the *Escherichia coli* FtsB/FtsL/FtsQ cell division complex. *BMC Struct. Biol. 11*, 28.
(30) MacKenzie, K. R., and Fleming, K. G. (2008) Association energetics of membrane spanning α-helices. *Curr. Opin. Struct. Biol. 18*, 412−419.
(31) Seddon, A. M., Curnow, P., and Booth, P. J. (2004) Membrane proteins, lipids and detergents: Not just a soap opera. *Biochim. Biophys. Acta 1666*, 105−117.
(32) Xiao Zhou, F., Cocco, M. J., Russ, W. P., Brunger, A. T., and Engelman, D. M. (2000) Interhelical hydrogen bonding drives strong interactions in membrane proteins. *Nat. Struct. Mol. Biol. 7*, 154−160.
(33) Choma, C., Gratkowski, H., Lear, J. D., and DeGrado, W. F. (2000) Asparagine-mediated self-association of a model transmembrane helix. *Nat. Struct. Mol. Biol. 7*, 161−166.
(34) Hendsch, Z. S., and Tidor, B. (1999) Electrostatic interactions in the GCN4 leucine zipper: Substantial contributions arise from intramolecular interactions enhanced on binding. *Protein Sci. 8*, 1381−1392.
(35) Renthal, R., Brancaleon, L., Pena, I., Silva, F., and Chen, L. Y. (2011) Interaction of a two-transmembrane-helix peptide with lipid bilayers and dodecyl sulfate micelles. *Biophys. Chem. 159*, 321−327.
(36) Tulumello, D. V., and Deber, C. M. (2011) Positions of Polar Amino Acids Alter Interactions between Transmembrane Segments and Detergents. *Biochemistry 50*, 3928−3935.
(37) Posokhov, Y. O., Merzlyakov, M., Hristova, K., and Ladokhin, A. S. (2008) A Simple "Proximity" Correction for FRET Efficiency Determination in Membranes Using Lifetime Measurements. *Anal. Biochem. 380*, 134−136.
(38) Doura, A. K., and Fleming, K. G. (2004) Complex interactions at the helix-helix interface stabilize the glycophorin A transmembrane dimer. *J. Mol. Biol. 343*, 1487−1497.
(39) Burgess, N. K., Stanley, A. M., and Fleming, K. G. (2008) Determination of Membrane Protein Molecular Weights and Association Equilibrium Constants Using Sedimentation Equilibrium and Sedimentation Velocity. In *Methods in Cell Biology* (Correia, J. J., and Detrich, H. W., Eds.) pp 181−211, Academic Press, New York.
(40) Gonzalez, M. D., Akbay, E. A., Boyd, D., and Beckwith, J. (2010) Multiple interaction domains in FtsL, a protein component of the widely conserved bacterial FtsLBQ cell division complex. *J. Bacteriol. 192*, 2757−2768.
(41) Masson, S., Kern, T., Le Gouëllec, A., Giustini, C., Simorre, J.-P., Callow, P., Vernet, T., Gabel, F., and Zapun, A. (2009) Central domain of DivIB caps the C-terminal regions of the FtsL/DivIC coiled-coil rod. *J. Biol. Chem. 284*, 27687−27700.
(42) Van den Berg van Saparoea, H. B., Glas, M., Vernooij, I. G. W. H., Bitter, W., den Blaauwen, T., and Luirink, J. (2013) Fine-mapping the Contact Sites of the *Escherichia coli* Cell Division Proteins FtsB and FtsL on the FtsQ Protein. *J. Biol. Chem. 288*, 24340−24350.
(43) D'Ulisse, V., Fagioli, M., Ghelardini, P., and Paolozzi, L. (2007) Three functional subdomains of the *Escherichia coli* FtsQ protein are involved in its interaction with the other division proteins. *Microbiology (Reading, U.K.) 153*, 124−138.
(44) Karimova, G., Dautin, N., and Ladant, D. (2005) Interaction network among *Escherichia coli* membrane proteins involved in cell division as revealed by bacterial two-hybrid analysis. *J. Bacteriol. 187*, 2233−2243.