SUPPLEMENTARY INFORMATION

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

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Supplementary Methods

1. N-terminal labeling of peptides

1.1 FITC labeling.
12 equivalents of N-ε-Fmoc-aminohexanoic acid (Fmoc-ε-Ahx-OH, AnaSpec) were coupled to the amino terminal of the peptide-resin overnight. After thorough washing, 20% Piperidine in DMF (Dimethylformamide) with 2% DBU (1,8-Diazabicyclo[5.4.0]undec-7-ene) was used to remove the Fmoc protecting group in two cycles in the MARS microwave at the UW Biotech center. 12 equivalents of FITC (Fluorescein isothiocyanate) 'isomer 1' (Sigma Aldrich) were mixed with 12 equivalents of PyBOP (Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) and resuspended in minimal volume of DMF, and then NMP (N-Methylpyrrolidone) was added dropwise while stirring until a homogeneous solution was formed. This mixture was added to the deprotected peptide-resin while agitating the resin, and DIPEA (N,N-Diisopropylethylamine) was added dropwise. The reaction was carried out at room temperature, in dark, for 24 hours, washed thoroughly, and repeated 4 times. Efficient labeling of the peptide was achieved by this 'brute force' method of labeling the peptides. FITC, due to its high hydrophobicity, was particularly hard to label the peptides with, and required more number of repeats for efficient labeling. For the FtsL peptide, 5-carboxyfluorescein (5-FAM) was used to efficiently label the peptide using the same method as above.

1.2 Coumarin labeling.
7-hydroxycoumarin-3-carboxylic acid (Anaspec) was coupled directly to the deprotected peptide-resin using the same technique described above, without the coupling of the Fmoc-ε-Ahx-OH linker. Addition of higher excess of DIPEA led to better labeling efficiencies for the coumarin labeled peptides. Frequent changes of reaction mixture for coumarin labeling minimized aggregation of the mixture to form an insoluble precipitate.

2. 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 procedure[1]. The calculations used have been listed below. It is important to note here that in the case of a labeled peptide, the absorbance of the peptide sample at 280 nm comprises of contributions from Trp and Tyr residues of both labeled and unlabeled peptides, as well as the absorbance of the fluorophore at 280 nm. To separate these components and achieve accurate quantification, 'correction factor' of the fluorophore (A_{280}/A_{max}) and accurate degree of labeling values need to be used. Correction factors for commonly used fluorophores are characterized along with their molar extinction coefficients at their λ_{max} values in aqueous buffers at a certain pH. However, these parameters change for the fluorophore in different solvent systems. Figure S1(a) shows a blue shift for the absorbance of FITC in TFE as compared to phosphate buffer, pH 9.0, where the molar extinction coefficient of FITC at 495nm is ~70,000 M^{-1} cm^{-1}. Various concentrations of FITC in TFE were scanned as shown in Figure S1(b). Figure S1(b) inset shows a plot of the new A_{max} (477nm) of FITC in TFE versus FITC concentration. The slope of the curve provided the new $\varepsilon_{Fluorophore}$ (~32690 $M^{-1}cm^{-1}$) at the new λ_{max} (477nm) of FITC in TFE, which was used for quantification of the FITC peptides. The behavior of 5-FAM was found to be the same as FITC in TFE. The same calculations were carried out for 7-hydroxycoumarin-3-carboxylic acid in TFE (data not shown).

2.1. Concentration of the fluorophore in the sample

$$\textbf{[Fluorophore]} = A_{max}/\varepsilon_{Fluorophore}\, l \quad \textbf{(1)}$$

where A_{max} is absorbance at λ_{max} of the dye
$\varepsilon_{Fluorophore}$ is extinction coefficient of the dye at λ_{max}
l is path length of the cuvette

2.2. Concentration of the peptide based on Trp absorbance

$$\textbf{[Peptide]} = A_{280} - (A_{max} * CF)/\varepsilon\, l \quad \textbf{(2)}$$

where A_{280} is absorbance at 280nm
CF is the correction factor that adjusts for absorbance at 280 nm by the fluorophore, and is given by A_{280} / A_{max}
ε is extinction coefficient of the peptide (calculated based on no. of Trp, and Tyr)

2.3. Calculation of the degree of labeling

$$\textbf{Percent labeling} = \textbf{[Fluorophore]/[Peptide] * 100} \quad \textbf{(3)}$$

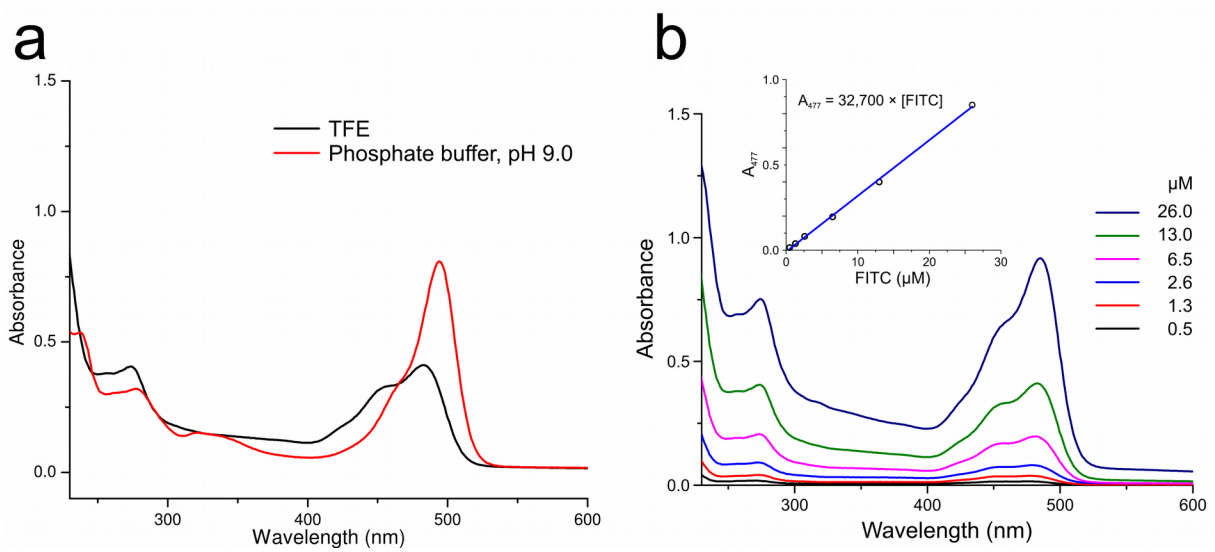

Figure S1. Characterization of FITC in TFE. a) Red curve shows absorbance scan of FITC in phosphate buffer pH 9.0, showing a λ_{max} of 495 nm. Black curve shows absorbance scan equal concentration of FITC in TFE, exhibiting a blue shift of the λ_{max} to 477nm. b) Absorbance scans of different concentrations of FITC in TFE. The slope of the absorbance plot at 477 nm, according to Beer's law, yielded the molar extinction coefficient of FITC in TFE.

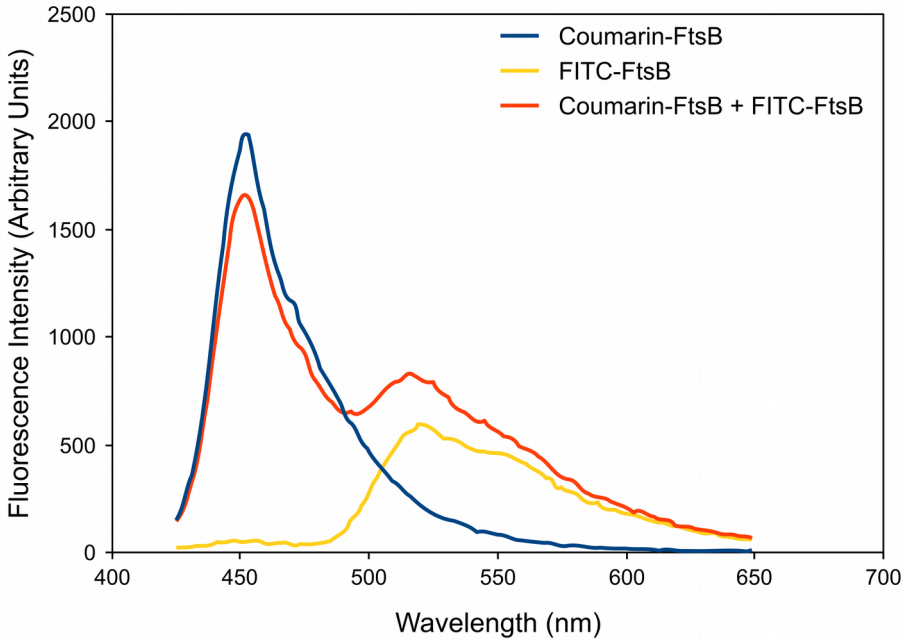

Figure S2. Fluorescence spectra of coumarin (donor) and FITC (acceptor) labeled peptides. Characteristic fluorescence scans of peptides in POPC in a 1:5000 peptide:lipid ratio. The excitation wavelength was fixed at 415 nm for maximum spectral overlap for the donor-acceptor pair. The emission scan was from 425 nm to 650 nm. The coumarin-FtsB+FITC-FtsB sample (orange curve) shows donor quenching compared to the coumarin-FtsB only sample (blue curve). The donor+acceptor pair also shows acceptor sensitization above the level of FITC-FtsB direct excitation at 415 nm (yellow curve), indicating FRET between the two FtsB peptides. Percentage FRET was calculated from decrease in the donor emission maxima at 450 nm.

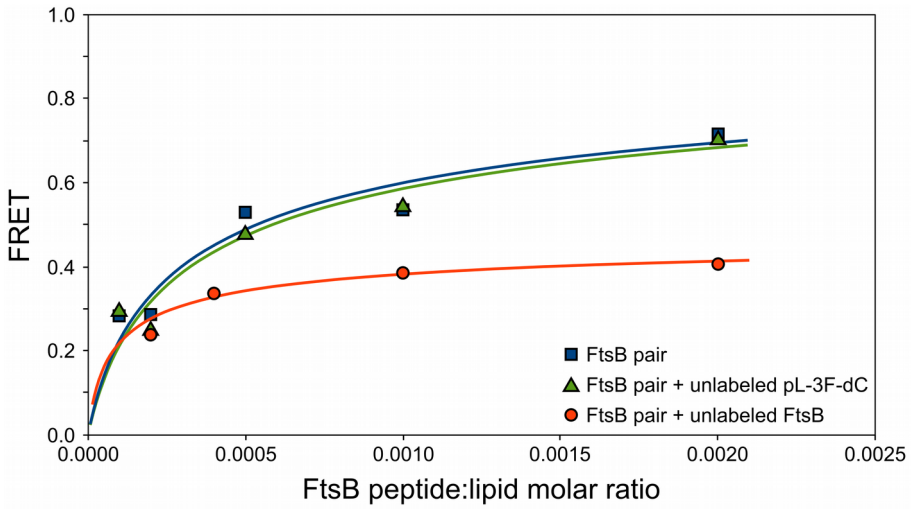

Figure S3. Effect of unlabeled control peptides on FtsB homo-FRET. Addition of equimolar amount of an unrelated monomeric peptide, pL-3F-dC (green triangles) to an FtsB FRET pair in POPC lipid does not shift the curve compared to the no-peptide control (blue squares). Addition of an equimolar amount of unlabeled FtsB reduces FRET in a manner that is consistent with a 50% decrease in FRET efficiency. All curves were fit assuming an FtsB monomer-dimer equilibrium. The “unlabeled FtsB” data set was fit by using total FtsB peptide (labeled + unlabeled) for concentration and a maximum FRET efficiency of 50%.

References

(1) Khadria, A., and Senes, A. (2013) Measurement of Transmembrane Peptide Interactions in Liposomes Using Förster Resonance Energy Transfer (FRET). *Methods Mol. Biol. Clifton NJ 1063*, 19–36.