Supplementary File 1

YejL is detectable in the periplasm
To check if YejL is detectable in the periplasm, although it does not carry the appropriate signal sequence, we have performed cellular fractionation studies. In the first set of the experiment, we overexpressed YejL in *E. coli* BL21DE3 cells. In the second experiment, we endogenously expressed YejL that was tagged with Yellow Fluorescent protein (YFP) using the *E. coli* strain SX1992 (obtained from the Coli Genetic Stock Center (CGSC)) (Supplementary Figure 1). In both experiments we observed YejL in the osmotic shock fluid (OSF), which represents the periplasmic fraction of the Gram-negative bacterial cells. In the over-expression experiment, YejL was observed in non-induced and induced samples, indicating leaky expression (Supplementary Figure 1A). YejL-YFP, which is endogenously expressed in *E. coli* SX1992, was observed in the OSF after 8 h at 37 °C (Figure 1B). We therefore conclude that although YejL is lacking an appropriate signal sequence, it is being targeted by an unknown mechanism at some levels into the periplasm. Once possibility is that YejL could co-localize in the cytoplasm with another protein that is targeted to the periplasm, and in this way, localizes in the periplasm. Further in-depth experiments would be needed to decipher this hypothesis.

YejL forms a higher weight oligomer in native PAGE
We further optimized our native PAGE sample preparation and protocol and can now show better quality native gels (Supplementary Figure 2). In the optimized native gels, it was more evident that no clear shift in size nor the formation of a band corresponding to the size of the YejM(191/241-586)-YejL complex was observed. The higher molecular weight population of YejL at around 66 kDa may be an artifact from YejL interacting with Coomassie Brilliant Blue G250 and/or the polyacrylamide matrix in the native gel, as our SEC-MALS experiments show YejL as a monomer in solution (see below, Supplementary Figure 3).

Absolute molecular mass determination by SEC-MALS did not reveal complex formation between YejL and YejM241-586
To determine the absolute molecular mass and detection of YejM241 and YejL oligomers in solution, we analyzed the proteins using size-exclusion chromatography coupled with multi-angle light scattering (SEC-MALS). SEC-MALS allows for separation of the sample mixture by size followed by light scattering intensity and concentration values of each fraction, from which the absolute molecular mass can be determined. Because the concentration is measured by the differential refractive index (dRI) and not by absorption at 280 nm, it is independent of the presence of aromatic residues in the protein sample; YejL in particular has a low content of aromatic residues.

In our SEC-MALS experiments the periplasmic domain YejM241-586 was not observed as a dimer at concentrations of 175µM (Supplementary Figure 3). However, this might not reflect local physiological concentration levels as well as the high concentrations needed during crystallization. We further analyzed whether we would detect YejM-YejL complex formation by SEC-MALS. If YejM and YejL form a stable complex, a fraction with molecular mass larger than YejM and YejL alone would be detectable. In our SEC-MALS experiment (Supplementary Figure 3), we did observe a change in absolute molecular mass between YejL alone (blue line), YejM241 alone (red line) and YejM:YejL in a 1:1 molar ratio (Magenta). However, the size difference between YejM241(red line) and in complex with YejL.
(magenta) is in the range of error for the molecular mass of YejM241 alone, given that the molecular mass of 29kDa (red line) is low compared to the calculated molecular weight of 41.1 kDa. If YejL is indeed a binding partner for YejM, we expect the binding affinity to be in a millimolar range, therefore we might not have found the correct concentration range and/or buffer conditions under which they bind in solution. We therefore reason that the interaction, if at all present, may be very weak.