

Supplementary Table 1. Anomalous data collection statistics

	Br-soak [‡]	Native ($\lambda = 1.31 \text{ \AA}$) [‡]	Native ($\lambda = 1.77 \text{ \AA}$) [*]
Data collection			
Space group	C2	C2	C2
Cell dimensions			
a, b, c (\AA)	93.92, 100.37, 75.15	90.69, 100.00, 75.6	93.73, 99.38, 74.71
α, β, γ ($^\circ$)	90, 112.74, 90	90, 106.61, 90	90, 112.72, 90
Wavelength (\AA)	0.9197	1.3106	1.7712
Resolution (\AA)	100–2.51 (2.60–2.51) ^a	100–2.50 (2.59–2.50) ^a	100–2.50 (2.59–2.50) ^a
R_{pim} (%)	9.2 (65.2)	3.0 (14.7)	4.0 (11.8)
$I/\sigma(I)$	9.1 (2.0)	13.8 (3.3)	22.0 (7.4)
$CC_{1/2}$	0.99 (0.30)	1.00 (0.95)	1.00 (0.96)
Completeness (%)	97.3 (88.8)	99.2 (97.7)	100 (100)
Redundancy	3.0 (2.4)	6.5 (5.4)	14.6 (12.7)

^{*} Merged from 3 native crystals grown in LCP.

[‡] From single crystal grown in LCP.

^a Values in parentheses are for highest-resolution shell.

Supplementary Note 1. Alignment of MurJ sequences. Positions shaded in red are identical in >90% of Gram-negative MurJ sequences in an alignment with 36 sequences, while those shaded gray are similar in >70% of the sequences. Asterisks (*) denote residues in MurJ_{TA} of which alanine mutants fail to complement (residue number is shown next to the asterisk). Carets (^) denote residues in *E. coli* MurJ of which cysteine mutants fail to complement upon MTSES treatment (Sham, L.-T. *et al.*, Science. 345(6193), 220–222, 2014). To generate this alignment, MurJ_{TA} from *Thermosipho africanus* (UniProt ID: B7IE18) was aligned with 36 representative Gram-negative MurJ sequences from the UniRef50 library (no representative sequence is more than 50% identical to another). MATE transporters PfMATE and NorM-NG were also included in the alignment.

List of sequences shown: MurJ-TA, *Thermosipho africanus* (UniProt ID: B7IE18); MurJ-EC, *Escherichia coli* (P0AF16); MurJ-PF, *Pseudomonas fluorescens* (G8PWV6); MurJ-VC, *Vibrio cholerae* (O34238); MurJ-LI, *Leptospira interrogans* (A0A0C5X9A5); MurJ-RS, *Rhodobacter sphaeroides* (Q3J5H7); PfMATE (PDB ID: 3VVN); NorM-NG (4HUK).