

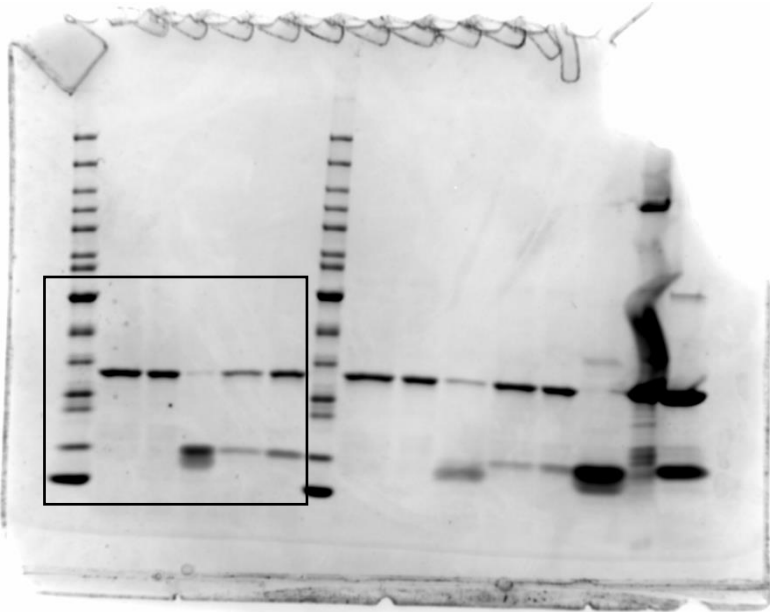
Strain	Description	Reference
TOP10	<i>E. coli</i> strain, F <sup>-</sup> <i>mcrA</i> Δ( <i>mrr</i> <i>hsdRMS</i> <i>mcrBC</i> ) Φ80 <i>lacZ</i> ΔM15 Δ <i>lacX74</i> <i>recA1</i> <i>araD139</i> Δ( <i>araleu</i> ) 7697 <i>galU</i> <i>galK</i> <i>rpsL</i> (StrR) <i>endA1</i> <i>nupG</i>	ThermoFisher Scientific
C43(DE3)	<i>E. coli</i> strain, F <sup>-</sup> <i>ompT</i> <i>hsdSB</i> (rB <sup>-</sup> mB <sup>-</sup> ) <i>gal</i> <i>dcm</i> (DE3))	Miroux and Walker, 1996 (ref <sup>34</sup> )
BL21(DE3)	<i>E. coli</i> strain: F <sup>-</sup> <i>ompT</i> <i>hsdSB</i> (rB <sup>-</sup> -mB <sup>-</sup> -) <i>dcm</i> <i>gal</i> (DE3)	ThermoFisher Scientific
MC4100	<i>E. coli</i> strain: F <sup>-</sup> <i>araD139</i> Δ( <i>argF-lac</i> )U169 <i>rpsL150</i> (StrR) <i>relA1</i> <i>flbB5301</i> <i>deoC1</i> <i>ptsF25</i> <i>rbsR</i>	Casadaban et al., 1976 (ref <sup>35</sup> )
Plasmid	Description	Reference
pPG1	CDS for C-terminally strep-II tagged <i>E. coli</i> CsgG (P0AEA2) in pASK-IBA3plus; <i>ColE1</i> ori, P <sub>tet</sub> <i>tetR</i> -operator, <i>ampR</i> resistance	Goyal et al., 2013 (ref <sup>17</sup> )
pNA152	modified pTRC99a with pDEST14 Gateway® cassette; <i>pBR322</i> ori, P <sub>trc</sub> <i>lacI</i> , <i>aadA</i> resistance	This study
pNA43	<i>E. coli</i> MC4100 <i>csgEFG</i> (with strep-II tagged <i>csgG</i> ) in pNA152	This study
pNA62	CDSs for C-terminally His-tagged <i>E. coli</i> CsgF and C-terminally strep-II tagged CsgG in pNA43	This study
pNA101	CDS for C-terminally His-tagged <i>E. coli</i> CsgF-protein (P0AE98) in pET22b; <i>pBR322</i> ori, P <sub>T<sub>lac</sub></sub> <i>lacI</i> , <i>ampR</i> resistance	This study
Sequence ID	Sequence	
Sequence 1	<u>GTMTFQFRNPNEFGGNPNNGAFLNLSAQAOQNSYKDPSYNDDFGIETPSALDNFTQAIQ</u> SQILGGLLSNINTGKPGRMVNTNDYIVDIANRDGQLQLNVTDRKTGTSTIQVSLQNN STDF	
Primer 1	CCCCCATATGGGAACCATGACTTTCAGTTCC	
Primer 2	CCCCGAATTCCTAATGGTGATGGTGATGGTGGTAAAAATCGGTTGAGTTATTTTG	
Primer 3	GGGGACAAGTTTGTACAAAAAAGCAGGCTACCTCAGGCGATAAAGCCATGAAAC GTTA	
Primer 4	GGGGACCACTTTGTACAAGAAAGCTGGGTGTTTAACTCATTTCGAACTGCGG GTGGCTCCAAGCGCTGG	
Primer 5	CAAATAACTCAACCGATTTTCATCACCATCACCATCACTAAGCCCCAGCTTCAT AAGG	
Primer 6	CCTTATGAAGCTGGGGCTTAGTGATGGTGATGGTGATGAAAATCGGTTGAGTTAT TTTG	
Primer 7	AGCCTGCTTTTTTGTACAAAC	
Primer 8	ATAAAAAATTGTTTCGGAGGCTGC	
Primer 9	GCACTCTCAGTACAATCTGCTC	
Primer 10	GTGAAATACCGCACAGATGCG	
3x_T3	CCCGGGTTTGGAAATTTGGAATTTAAGCTT	
3x_T4	CCCGGGTTTGGAAATTTGGAATTTAAGCTT	
3x_T5	CCCGGGTTTGGAAATTTTGGAAATTTTAAAGCTT	
3x_T6	CCCGGGTTTTTGGAAATTTTTGGAAATTTTTAAGCTT	
3x_T7	CCCGGGTTTTTGGAAATTTTTTGGAAATTTTTTAAAGCTT	
3x_T8	CCCGGGTTTTTGGAAATTTTTTGGAAATTTTTTAAAGCTT	
3x_T9	CCCGGGTTTTTGGAAATTTTTTGGAAATTTTTTAAAGCTT	

**Supplementary Table 1: Strains, plasmids and sequences used in this study.** The underlined sequence in sequence 1 corresponds to the CsgF constriction peptide.

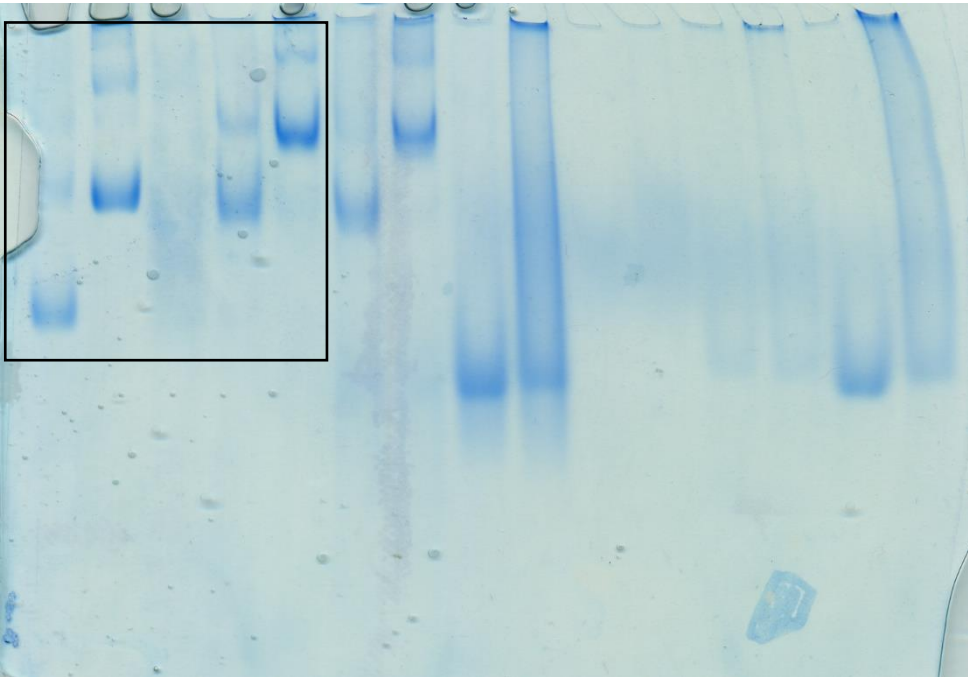
CsgG:CsgF (EMDB-10206) (PDB 6SI7)	
<b>Data collection and processing</b>	
Magnification	130.000
Voltage (kV)	300
Electron exposure (e <sup>-</sup> /Å <sup>2</sup> )	56
Defocus range (μm)	-1 to -3
Pixel size (Å)	1.07
Symmetry imposed	C9
Initial particle images (no.)	750.000
Final particle images (no.)	62.000
Map resolution (Å)	3.4
FSC threshold	0.143
Map resolution range (Å)	11.6-3.4
<b>Refinement</b>	
Initial model used (PDB code)	4UV3
Model resolution (Å)	3.3
FSC threshold	0.143
Model resolution range (Å)	
Map sharpening <i>B</i> factor (Å <sup>2</sup> )	-135
Model composition	
Non-hydrogen atoms	19476
Protein residues	2502
Ligands	0
<i>B</i> factors (Å <sup>2</sup> )	
Protein	52.85
Ligand	-
R.m.s. deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.805
Validation	
MolProbity score	2.15
Clashscore	12.95
Poor rotamers (%)	0
Ramachandran plot	
Favored (%)	90.81
Allowed (%)	9.19
Disallowed (%)	0

**Supplementary Table 2: Cryo-EM data collection, refinement and validation statistics.**

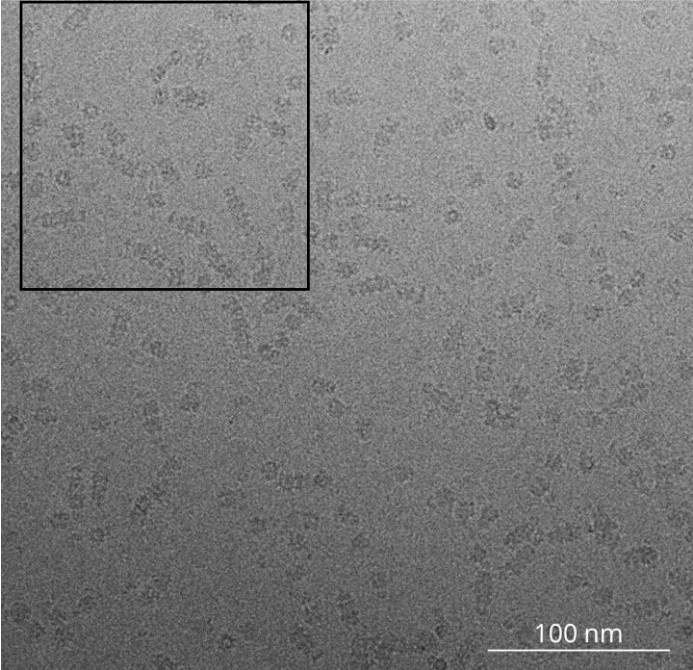
SDS-PAGE Figure 1b; cropped lanes used for publication are boxed.



Native PAGE Figure 1c; cropped area used for publication is boxed.



Complete EM micrograph used in Figure 1d; Region used for publication is boxed.



SDS-PAGE Extended Data Figure 1a; Cropped lanes used for publication are boxed.

