TABLE S1 MATCH/MatchScan identification of ExbD affinity-selected Ph.D.-12 and Ph.D.-C7C peptides aligned to ExbD sequence

Peptide[1]	Peptide MATCH Score[2]	Scoring window (residues)	Alignment position[3]
STPQPSLFSYPV	20	5	54
STGTPRA	17	6	52
K**STSQPE**	16	6	52
STPQVYNVFYAP	16	4	54
PPYLSTR	16	7	63
FHSHWPSM**ADNS**	16	4	70
TRTPQHS	15	5	53
K**STSQP**E	15	5	54
SSKV**LPSSFFT**R	15	7	49
PPYLSHL	15	5	63
STGTPRA	14	6	54
F**SISTLQ**SAPTR	14	6	52
HHRAHNS**HLSVR**	14	5	65
HH**TTST**G	13	4	52
TTTPTLH	13	4	53
NDPGRLR**VPVST**	13	5	49
AP**TTQTP**PINWK	13	5	52
LSTRAPL	13	5	66
HSIFY**PIYL**PSQ	13	4	63
FPHY**PVST**LYSL	12	4	50
PY**SAKA**H	12	4	67

[1] Scoring window residues in **bold**.

[2] Score of aligned residues based on modified BLOSUM62 matrix.

[3] First residue of scoring window aligned to protein sequence.

TABLE S2 MATCH/MatchScan identification of TonB affinity-selected Ph.D.-12 and Ph.D.-C7C peptides aligned to ExbD sequence

Peptide[1]	Peptide MATCH Score[2]	Scoring window (residues)	Alignment position[3]
N**STSSPQ**	21	6	52
LPASWHP	16	4	49
SH**SNTTQTRP**SD	16	8	53
SMNTFQP	15	7	52
N**PTPEK**R	15	5	58
SHA**LPLTWST**AA	15	7	49
NT**IPMHTST**HTI	15	7	49
IH**PASQS**RQNTT	15	5	50
AALGTY**STHTP**T	15	5	52
S**HLPAA**L	14	5	48
HLPTSSLFDTTH	14	6	48
K**TSLPR**L	13	5	46
RTF**DLPA**	13	4	48
PQPKTYQ	13	4	56
HG**LPVTT**RGAFG	13	5	49
TKTV**AQTTT**SIS	13	5	51
KLVDE**SSTS**PLS	13	4	51
L**TQTP**TR	12	4	53
HS**NLPT**KRPTSL	12	4	48

[1] Scoring window residues in **bold**.

[2] Score of aligned residues based on modified BLOSUM62 matrix.

[3] First residue of scoring window aligned to protein sequence.

TABLE S3 MATCH/MatchScan identification of ExbD affinity-selected Ph.D.-12 and Ph.D.-C7C peptides aligned to TonB sequence

Peptide[1]	Peptide MATCH Score[2]	Scoring window (residues)	Alignment position[3]
RH**SEPISVFYIT**	21	10	42
FHETWPARVSYL	21	10	125
QTTAWWG**APARL**	20	5	129
FH**SSTPTAPPQK**	20	10	135
S**PIYVTWV**PTAL	18	7	44
TNTAWTS	18	7	137
STNPAALYSDYS	18	9	127
L**SPARTT**	16	6	129
NG**LTSS**RPWSFL	16	4	133
R**PAPNQ**T	15	5	39
TVYWITP**PALPI**	15	5	41
H**PIYVT**YYPDPS	15	5	44
RHYEPL**SRVSSS**	15	6	131
KIY**PITLT**YLAP	14	5	44
I**SQPI**RQ	13	4	42
FHESW**PSPA**GGR	13	4	39
QTTAWWG**APAR**L	13	4	40
SHHW**EPIS**SPLR	13	4	43
T**MTGST**T	13	5	133
MTSSGML	13	4	133
HLLMKPP**QTSPA**	13	5	127
FSISTLQ**SAPTR**	13	5	128
QMMQ**TSSS**PPTV	13	4	134
P**HPIS**KQ	12	4	43
QTNSQ**HPIS**ALR	12	4	43
DR**APGR**T	12	4	129
STGTPRA	12	4	136

[1] Scoring window residues in **bold**.

[2] Score of aligned residues based on modified BLOSUM62 matrix.

[3] First residue of scoring window aligned to protein sequence.

TABLE S4 Candidate stoichiometries, their agreement with the SEC-MALLS-derived molecular mass and calculated pI of their soluble residues[1]

Candidate stoichiometry (ExbB–ExbD–TonB)	MW Agreement (%)[2]	Calculated pI (soluble residues)[3]
4–1–1	92	7.85
3–1–2	87	8.97
3–2–1	88	6.68
4–2–0	-	6.23

[1] Soluble residues (inclusive) of ExbB: 1–22, 42–131, 152–177, 198–244; ExbD: 1–22, 42–151; TonB: 1–9, 33–265.

[2] Agreement between the derived molecular mass of each candidate's stoichiometry and the theoretical mass.

[3] Isoelectric points calculated using soluble residues of each protein and using the program ProtParam from ExPASy.

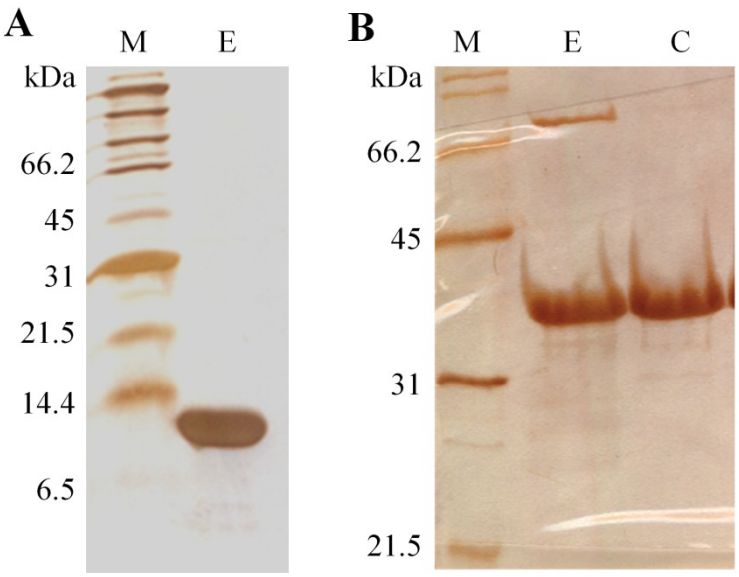

FIG S1 SDS-PAGE analysis of purified periplasmic domains of ExbD and TonB. (A) His_6-tagged $ExbD_{43-141}$ following elution (E) from Ni^+-NTA chromatography. (B) His_6-tagged $TonB_{33-239}$ following elution (E) from Ni^+-NTA chromatography and, (C) cation exchange chromatography.

A

MGNNLMQTDLSVWGMYQHADIVVKCVMIGLILASVVTWAIFFSKSVEFFNQKRRLK
REQQLLAEARSLNQANDIAADFGSKSLSLHLLNEAQNELELSEGSDDNEGIKERTSFR
LERRVAAVGRQMGRGNGYLATIGAISPFVGLFGTVWGIMNSFIGIAQTQTTNLAVVA
PGIAEALLATAIGLVAAIPAVVIYNVFARQIGGFKAMLGDVAAQVLLLQSRDLDLEAS
AAAHPVRVAQKLRAG

B

MAMHLNENLDDNGEMHDINVTPFIDVMLVLLIIFMVAAPLATVDVKVNLPASTSTPQ
PRPEKPVYLSVKADNSMFIGNDPVTDETMITALNALTEGKKDTTIFFRADKTVDYETL
MKVMDTLHQAGYLKIGLVGEETAKAK

C

MTLDLPRRFPWPTLLSVCIHGAVVAGLLYTSVHQVIELPAPAQPISVTMVTPADLEPP
QAVQPPPEPVVEPEPEPEPIPEPPKEAPVVIEKPKPKPKPKPKPVKKVQEQPKRDVKPV
ESRPASPFENTAPARLTSSTATAATSKPVTSVASGPRALSRNQPQYPARAQALRIEGQ
VKVKFDVTPDGRVDNVQILSAKPANMFEREVKNAMRRWRYEPGKPGSGIVVNILFKI
NGTTEIQ

FIG S2 LC-MS/MS confirms identities of ExbB, ExbD and TonB. (A) ExbB was identified with 16 unique peptides providing 55% coverage. (B) ExbD was identified with 13 unique peptides providing 67% coverage. (C) TonB was identified with 14 unique peptides providing 55% coverage. Sequences highlighted in yellow indicate the identified peptides. Green residues represent detected oxidation of methionine.

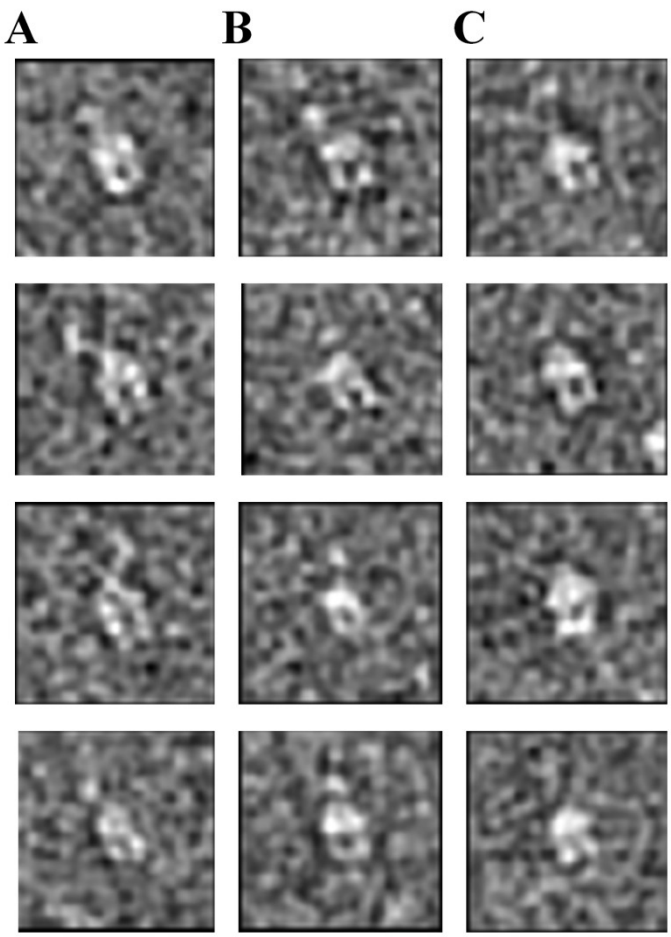

FIG S3 Gallery of single particles used for EM image analysis. DDM-solubilized $ExbB_4$–$ExbD_1$–$TonB_1$ single particles were aligned and low-pass filtered to 15 Å for clarity. (A) Gallery of particles showing the extensive periplasmic dimerization. (B) Gallery of single particles showing the distal periplasmic dimerization. (C) Gallery of single particles with no observable periplasmic dimerization.

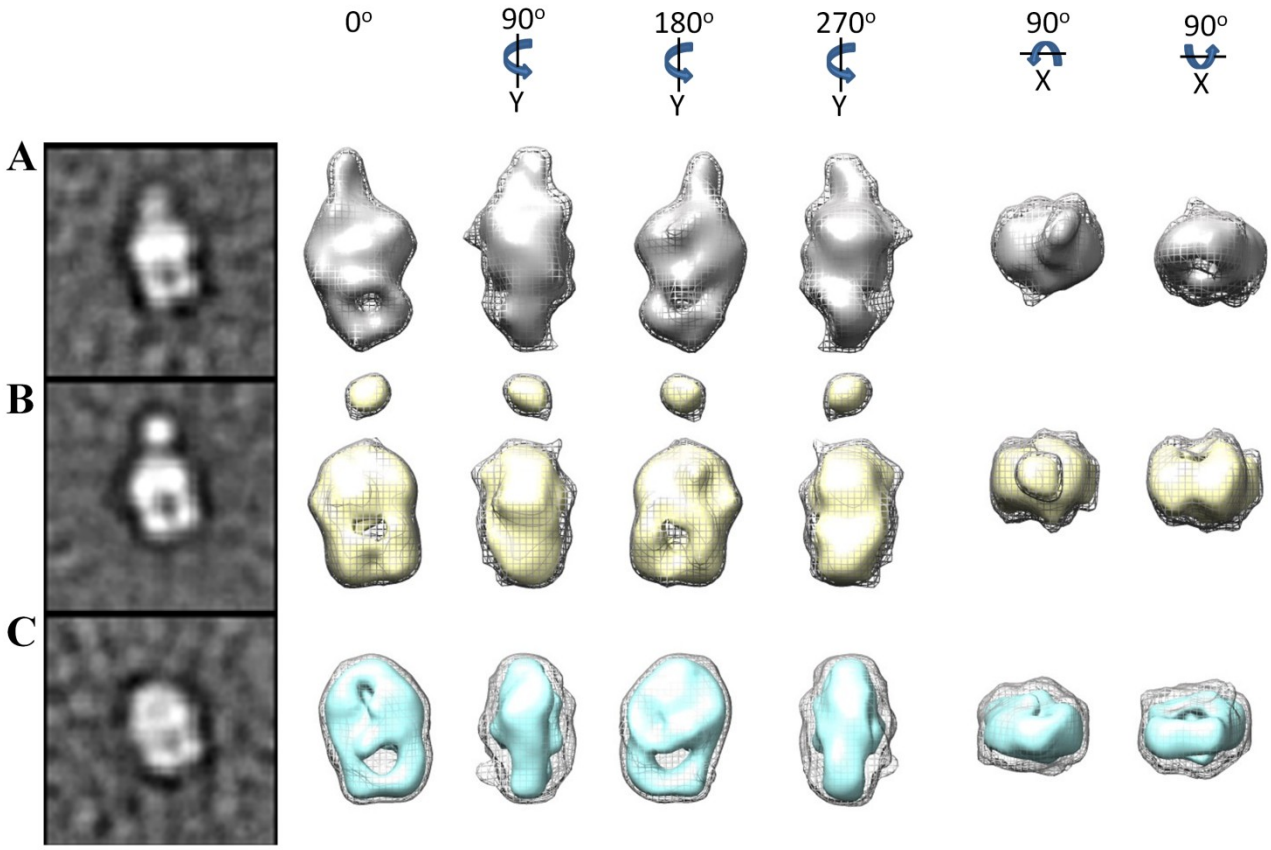

FIG S4 Initial 3D models of the $ExbB_4$–$ExbD_1$–$TonB_1$ complex by RCT. 2D averages of untilted particles and their 3D RCT models using tilted particles show the extensive periplasmic dimerization (A), distal periplasmic dimerization (B) and unobserved dimerization (C) conformational states of the $ExbB_4$–$ExbD_1$–$TonB_1$ particles. Initial 3D models were reconstructed using the in-plane rotation found by 2D classification combined with 60° tilt particles. All rotations are relative to 0°. The mesh contour represents volumes of ~230 kDa, with the inner volume threshold decreased to display features.

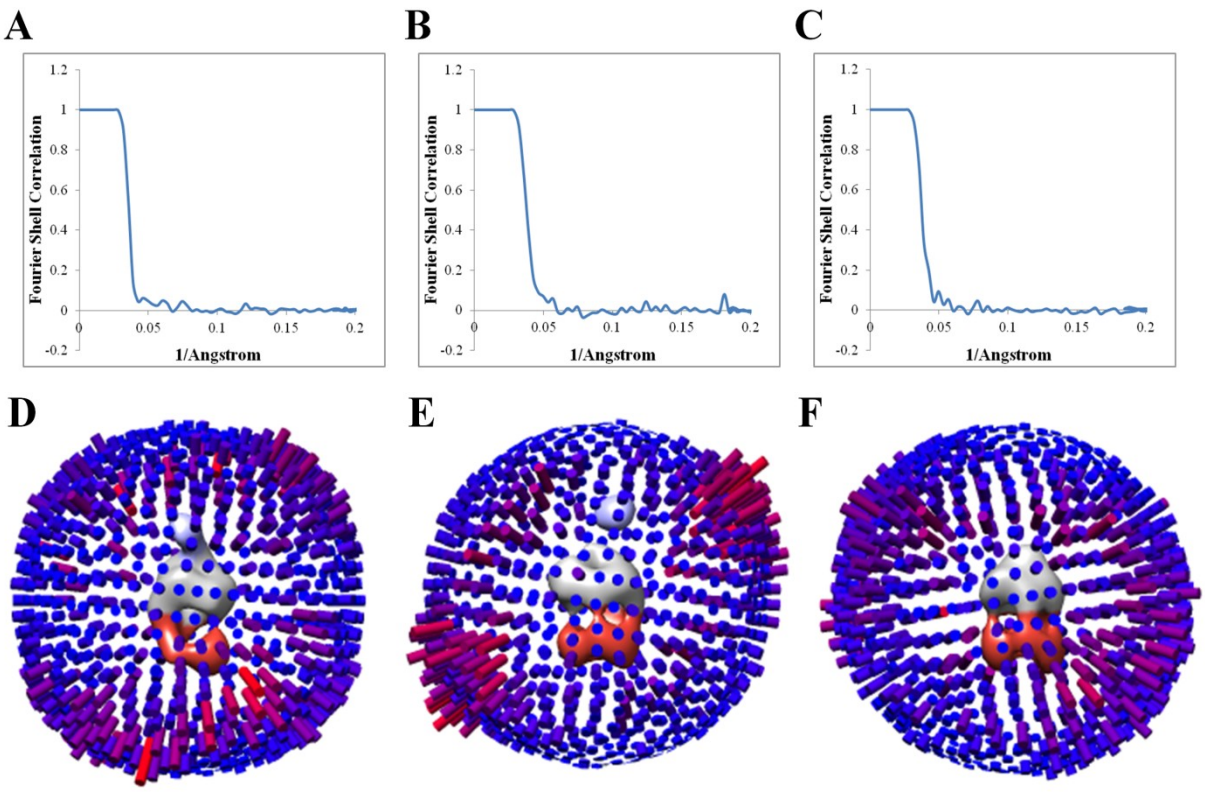

FIG S5 Metrics of 3D volume refinement. Gold standard Fourier shell correlation curves of the final 3D volumes representing the $ExbB_4$–$ExbD_1$–$TonB_1$ complex in three conformations. Using the 0.143 criterion, the resolutions of the extensive- (A), distal- (B) and unobserved (C) dimerization volumes are 28, 23 and 23 Å, respectively. Angular distribution plots showing complete angular coverage of the extensive- (D), distal- (E) and unobserved (F) dimerization volumes. The intensity of each point represents single particles of the same orientation, as determined with RELION refinement.

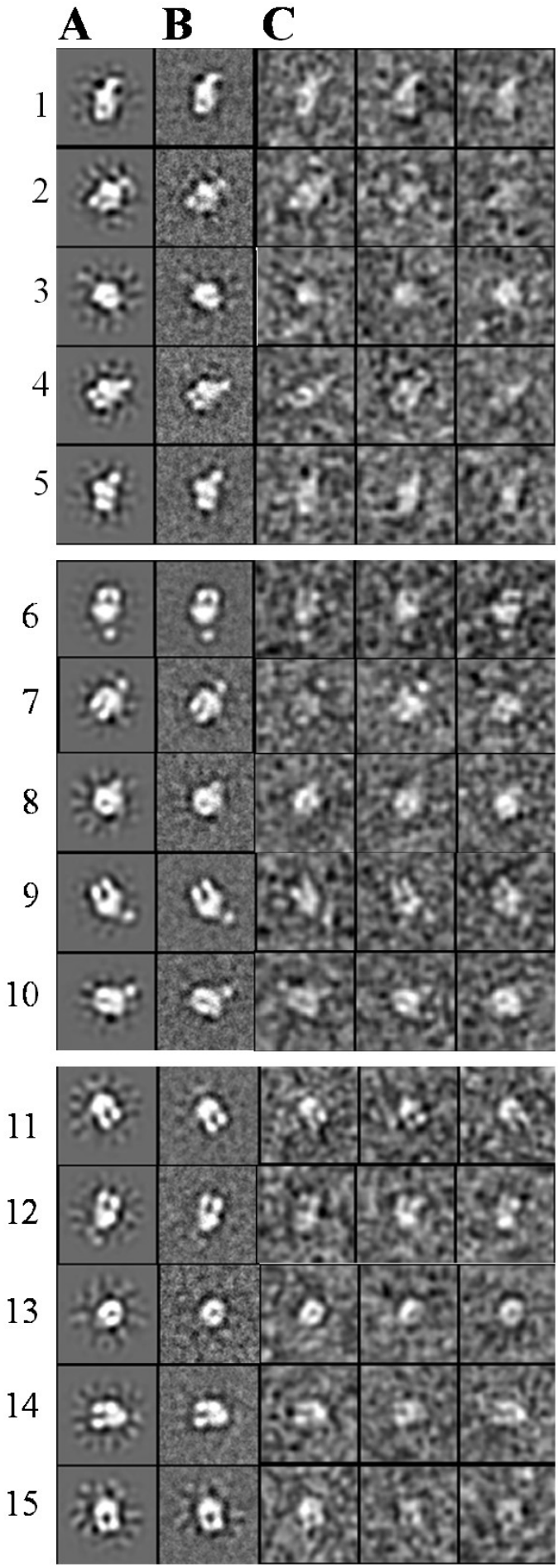

FIG S6 Comparison of final 3D volume reprojections to 2D class averages and aligned single particles. (A) Reprojections of characteristic views of three conformations: (1–5) extensive-dimerization; (6–10) distal-dimerization; and (11–15) unobserved dimerization. (B) Class averages corresponding to the same orientations as the volume reprojections. (C) Galleries of aligned single particles composing each class average, low-pass filtered to 15 Å for clarity.