Structural and molecular determinants for the interaction of ExbB from *Serratia marcescens* and HasB, a TonB paralog.

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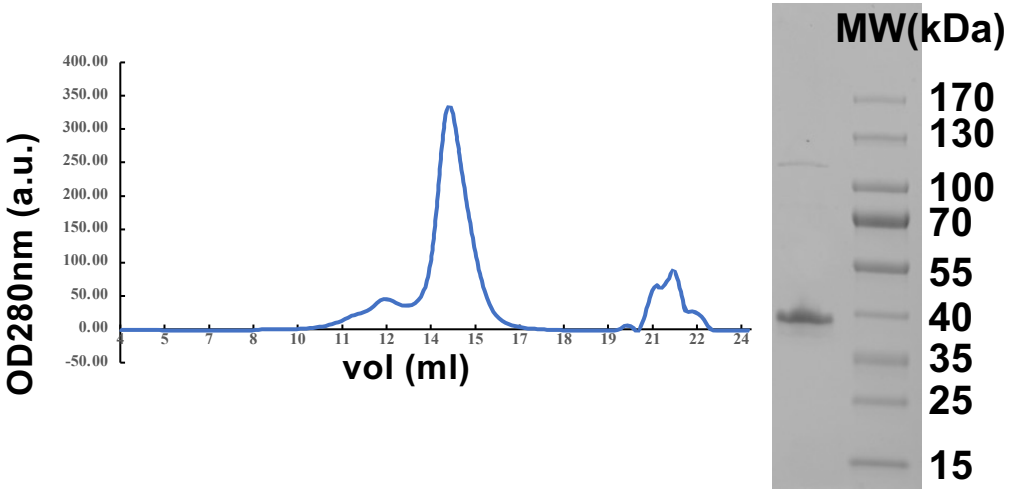
This file contains the following items:

supplementary Figures S1 to S15
supplementary Tables 1 and 2

Other supplementary information include

supplementary Data 1 (separate file) associated to Figure 3D
supplementary Data 2 (separate file) associated to Figure 5A

a



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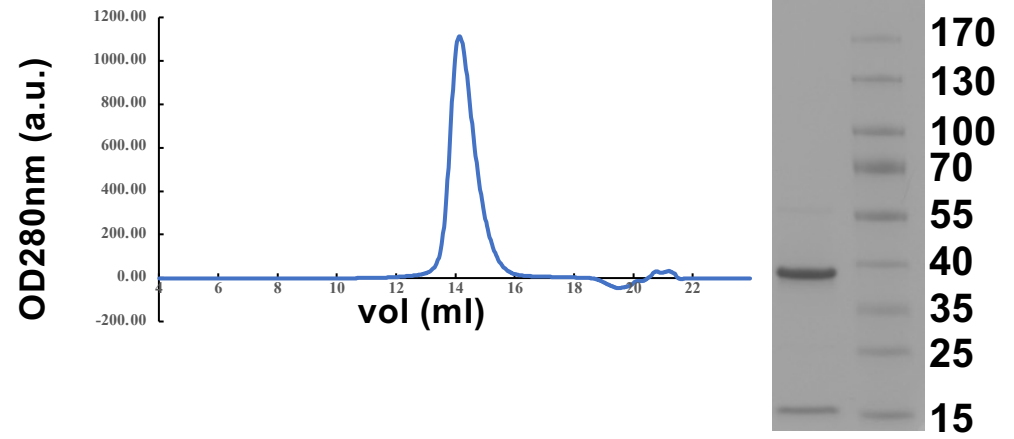
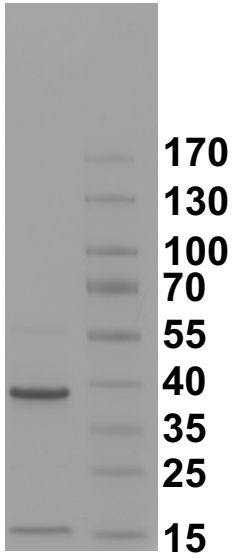
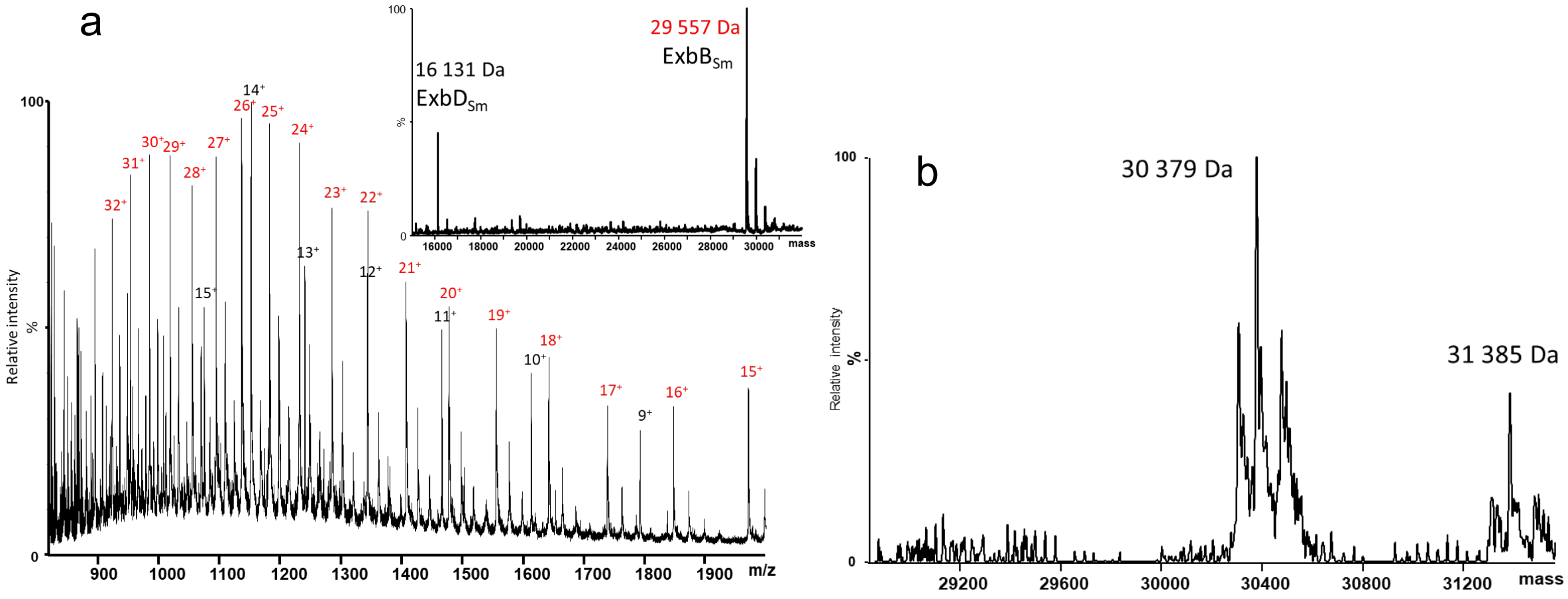
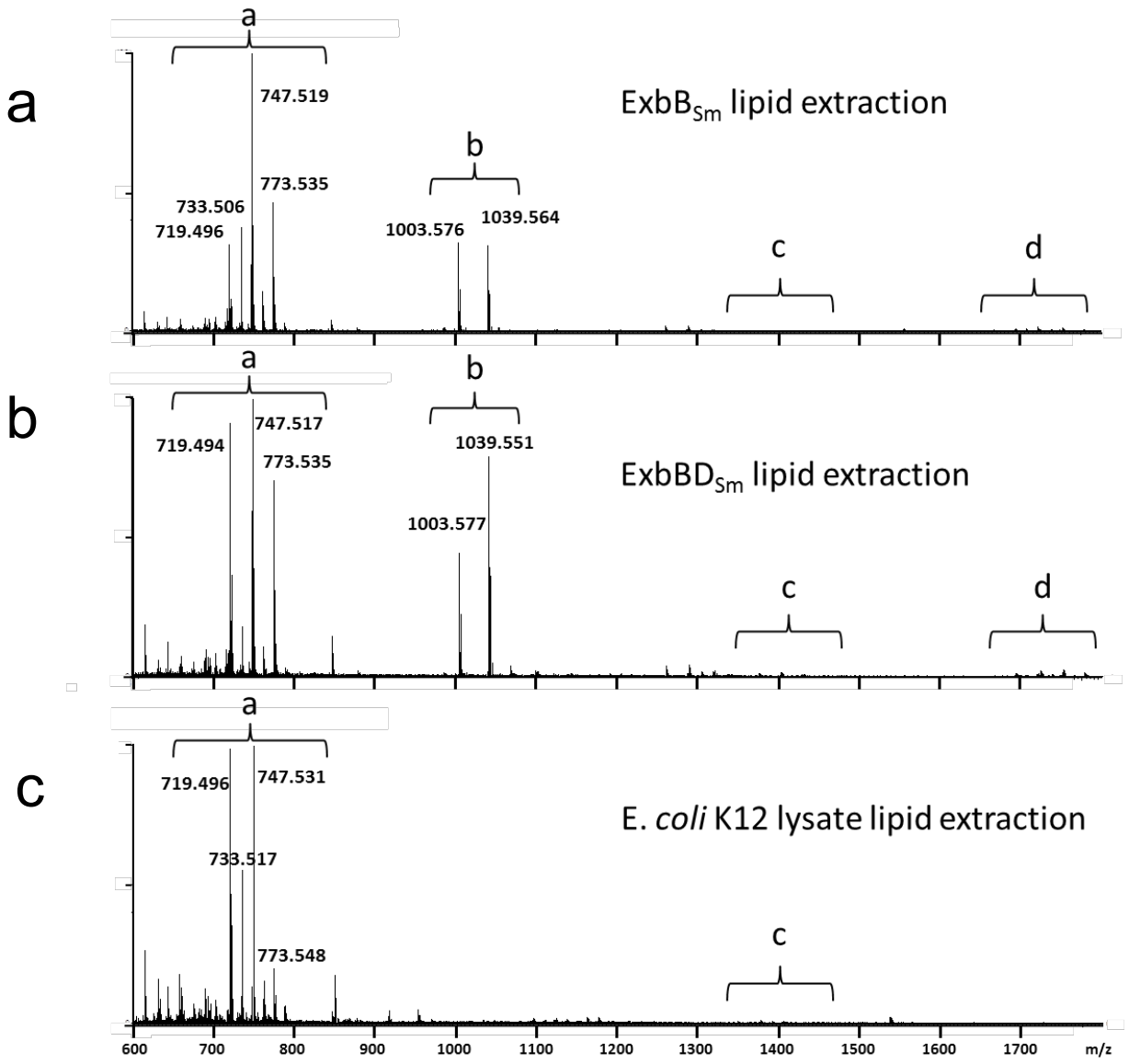




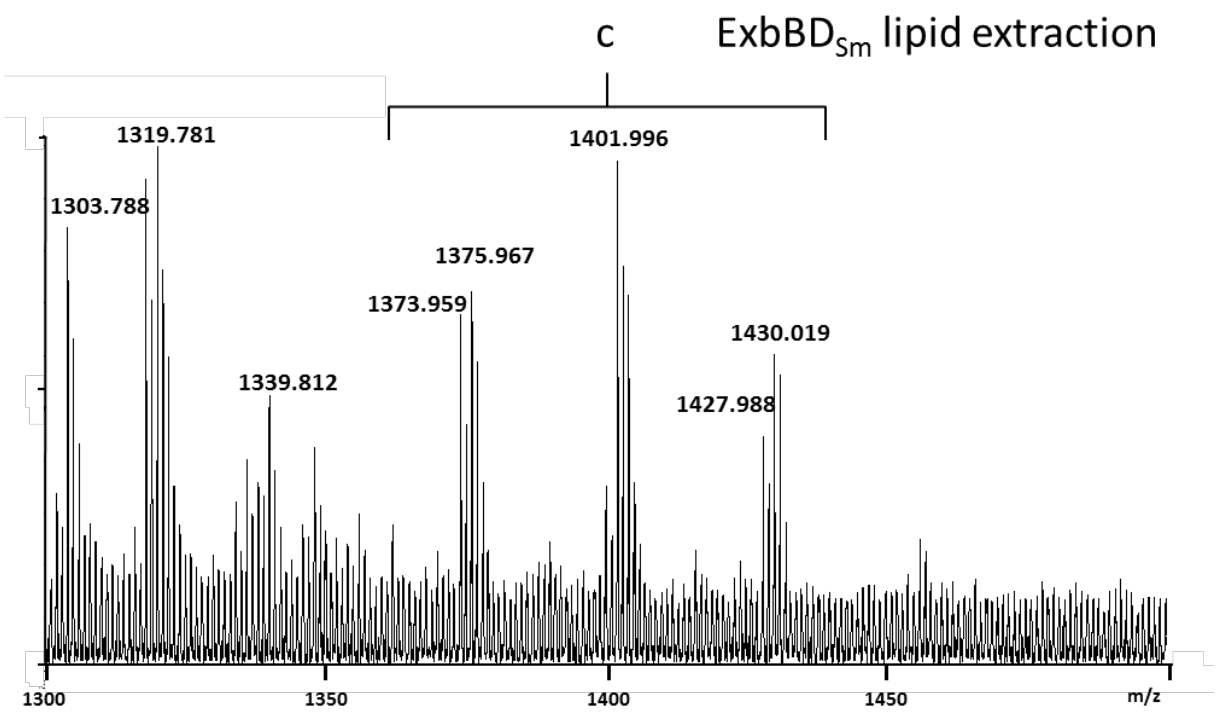
Supplementary Figure S1: Homogeneity assessment of ExbB and ExbBD. Size exclusion chromatography profiles of representative purification of respectively $ExbB_{Sm}$His6 (a) and $ExbBD_{Sm}$His6 (b) on a Superose 6 10/300 column. The 280nm absorbance is plotted as a function of elution volume. A Coomassie-stained gel of the pooled peak fractions is shown on the right of each profile together with a molecular weight ladder on the right (respectively 15, 25, 35, 40, 55, 70, 100, 130 and 170kDa). The faint band present in the Exb B_{Sm}His6 sample above 100kDa was identified as AcrB using mass spectrometry.



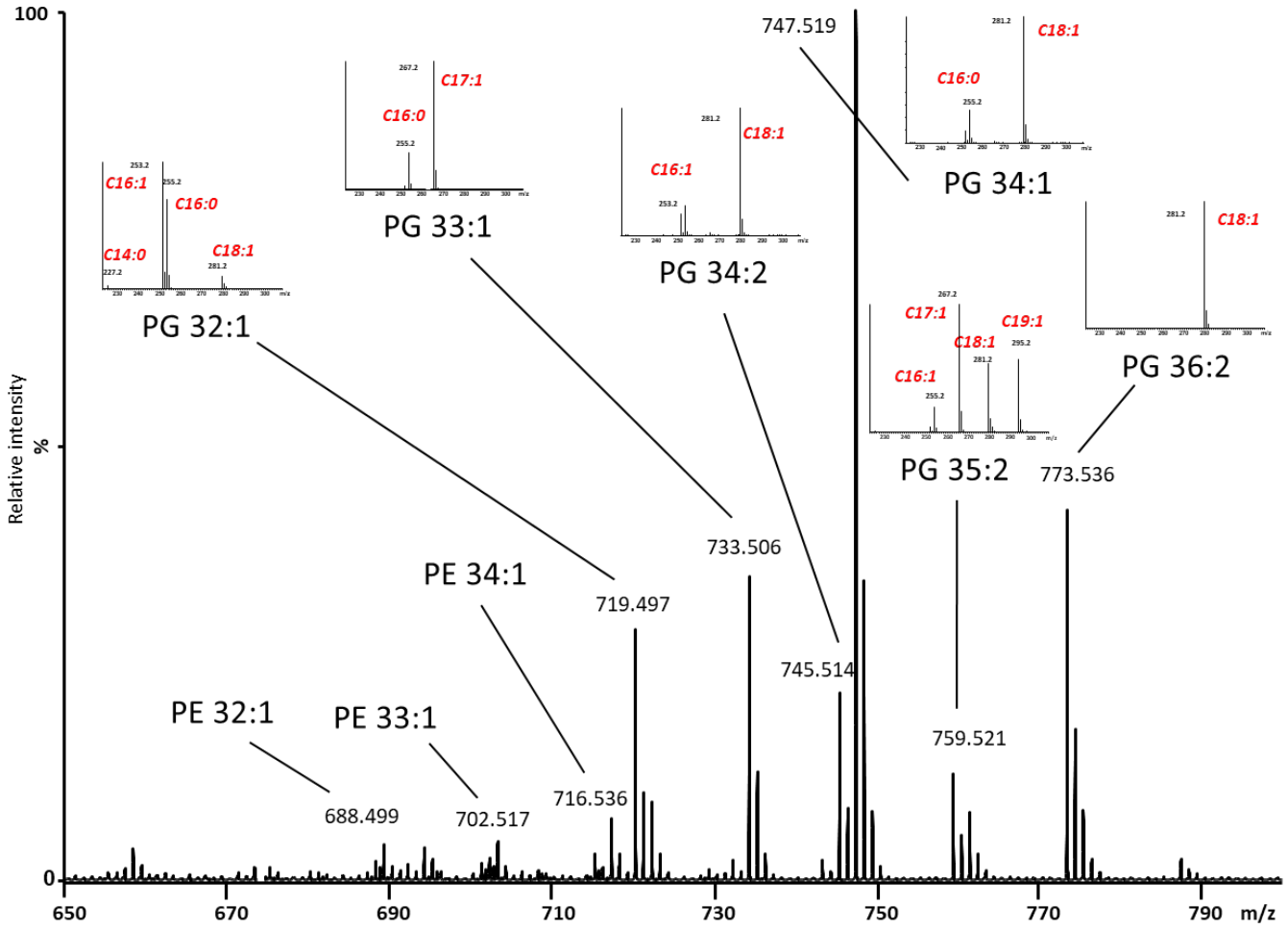
Supplementary Figure S2: Mass spectra of full-length proteins used in this study. a. MS spectrum (deconvoluted data in insert) of the ExbBD$_{Sm}$ complex in denaturing conditions (Mr$_{theoretical}$ ExbB$_{Sm}$ = 29 557 Da after signal peptide removal, Mr$_{theoretical}$ ExbD$_{Sm}$ = 16 162 Da after initial Met removal). b. Deconvoluted mass spectrum of purified ExbB$_{Sm}$ (Mr$_{theoretical}$ = 30 380 Da) showing the presence of the protein and an adduct with LMNG (Mr$_{theoretical}$ ExbB$_{Sm}$ + LMNG =31 385 Da).



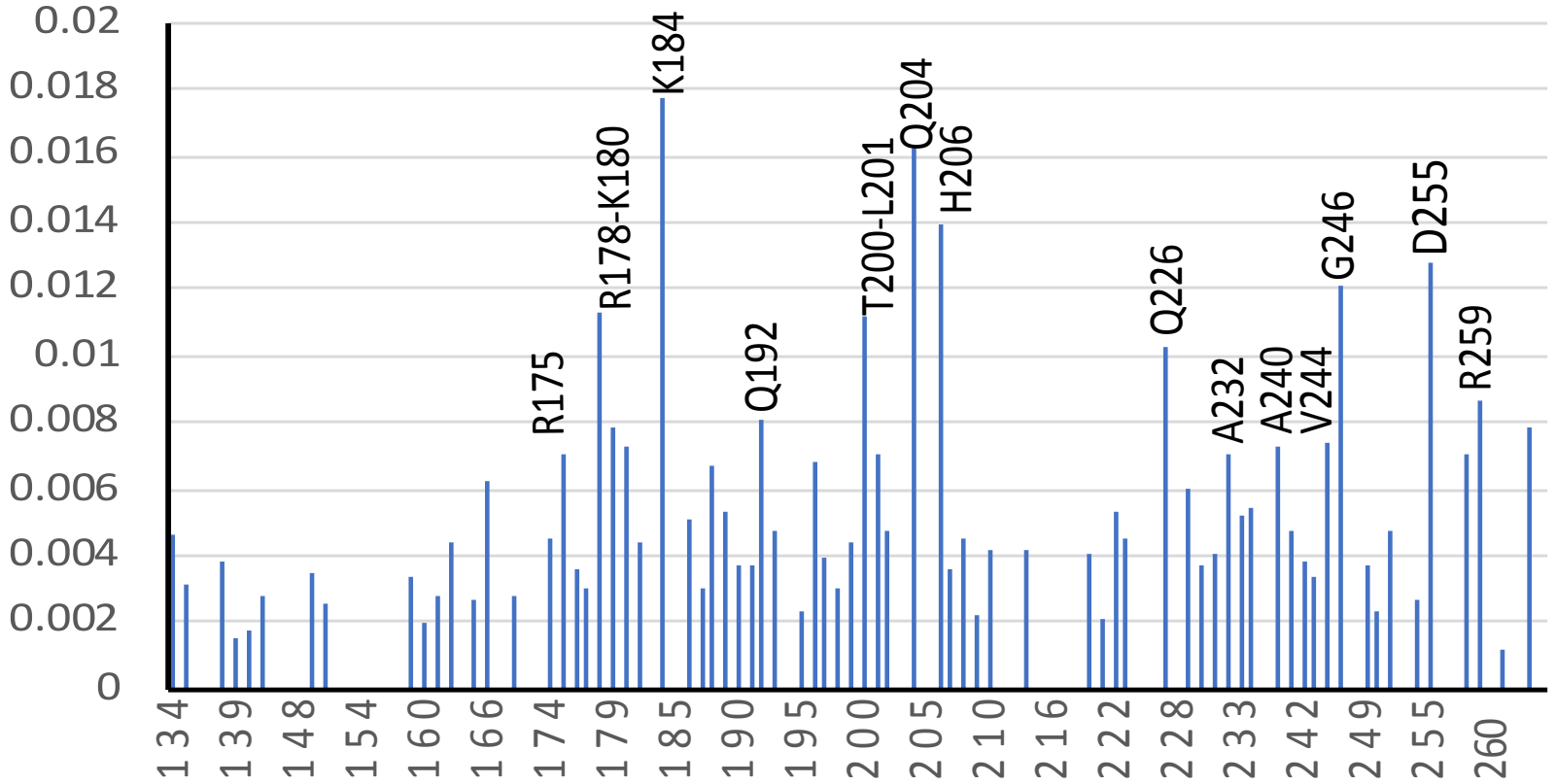
Supplementary Figure S3: Mass spectrometry analysis of lipid extracts for the following samples: a. ExbB$_{Sm}$ b. ExbBD$_{Sm}$ and $_{c}$. *E. coli* lysate. The (a) area corresponds to PE (phosphatidylethanolamine) and PG (Phosphatidylglycerol) derivatives; (b) to LMNG; (c) to cardiolipins and (d) to PE/PG LMNG adducts.



Supplementary Figure S4: Zoom of Figure S3B on the (c) area showing the different cardiolipins.

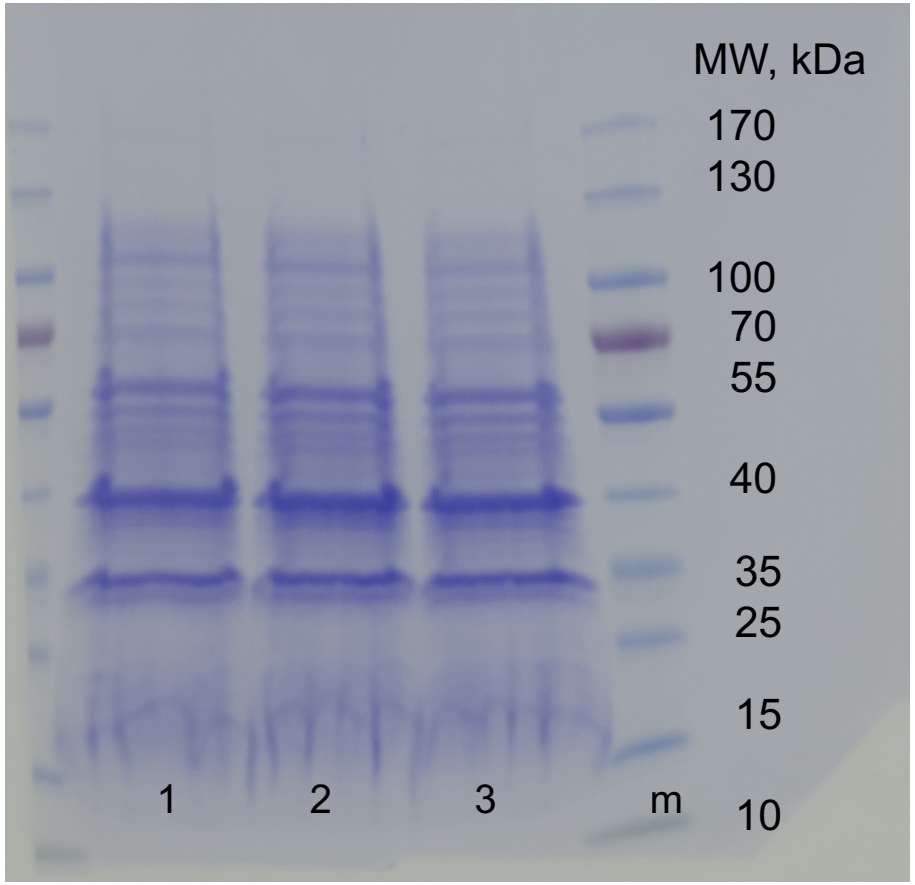


Supplementary Figure S5: Zoom of Figure S3A on the (a) area including CID spectra of the most intense ions in inserts. PE: phosphatidylethanolamine, PG: phosphatidylglycerol, the number of carbons and insaturations are also indicated.



Supplementary Fig S6: Histogram showing the chemical shift perturbation (CSP) values of backbone amide signals of $HasB_{CTD}$ (0.15 mM in 50mM sodium phosphate, pH 7, 50 mM NaCl) in the presence of the $ExbB_{Sm}$ 1-44 peptide, as a function of residue numbers. The protein/peptide ratio was 1:10. Residues showing CSP higher than 0.007 are considered for analysis, except the last residue.

a



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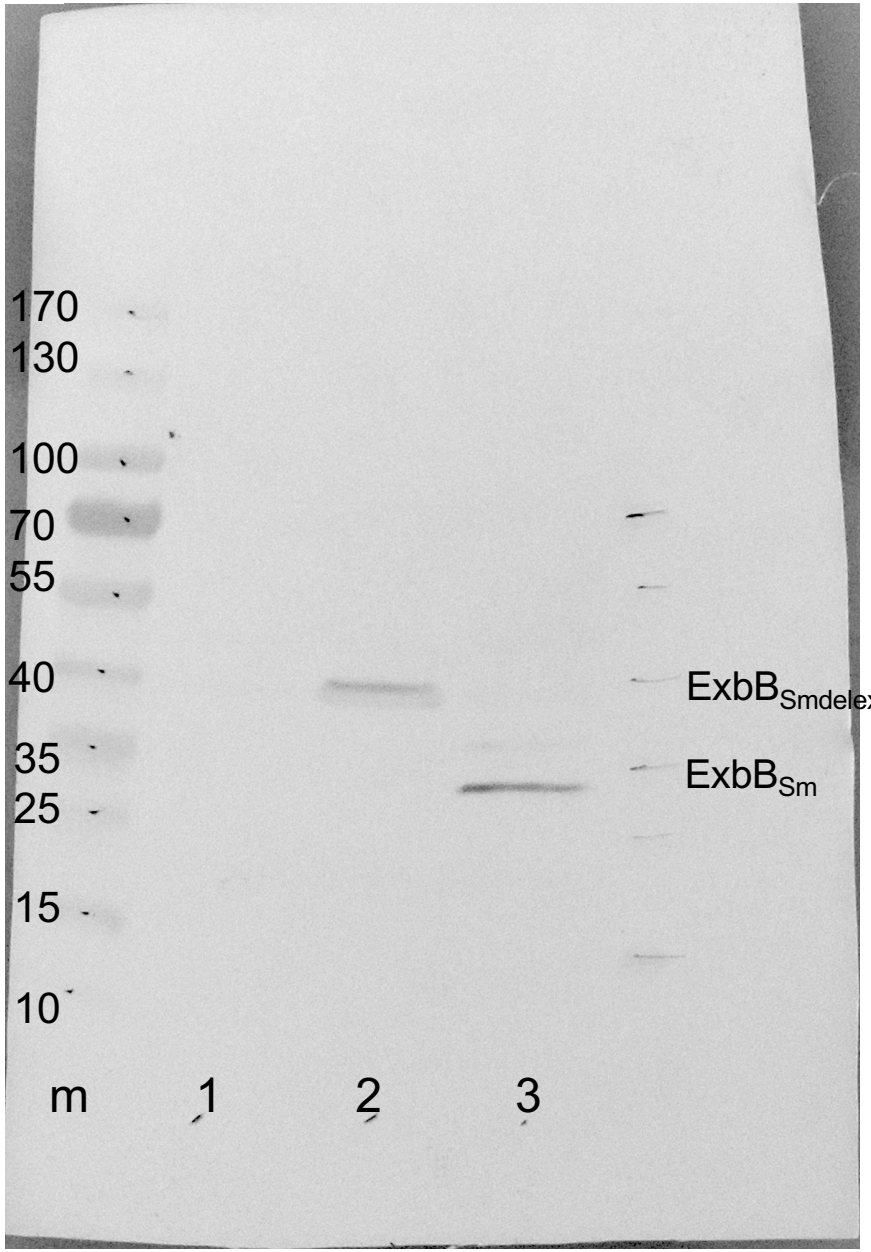
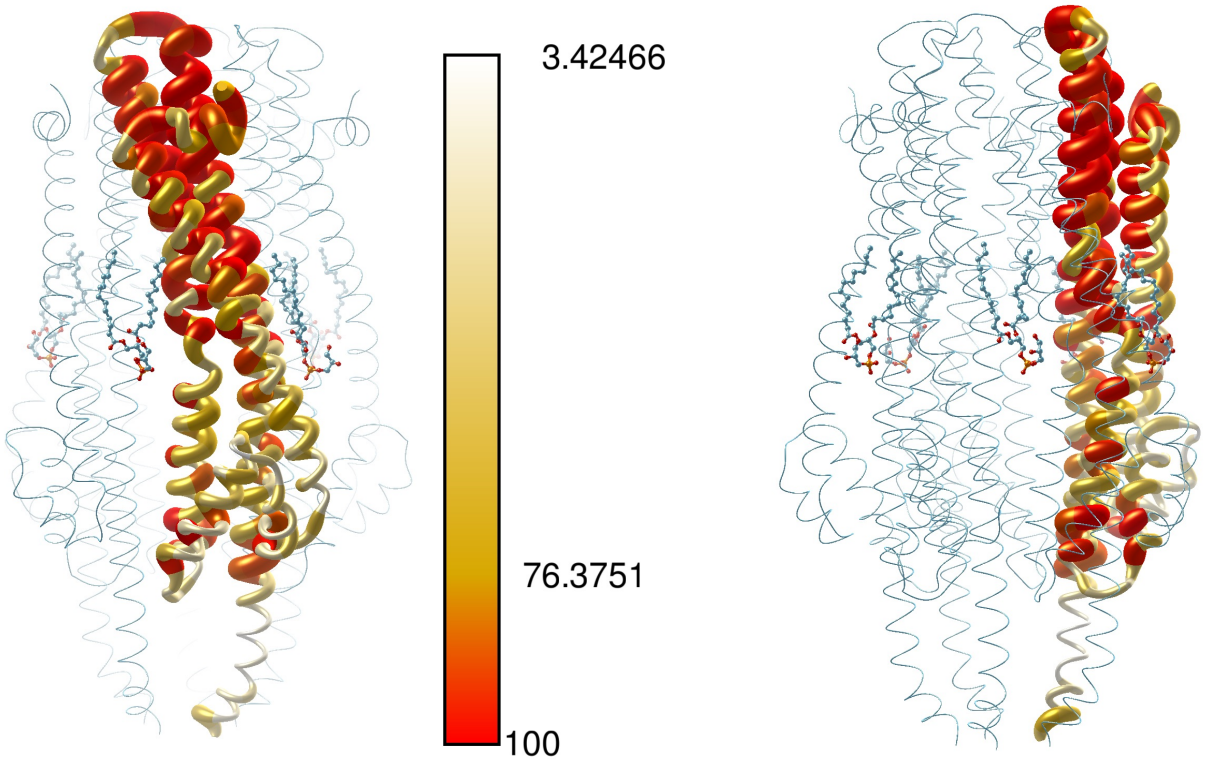
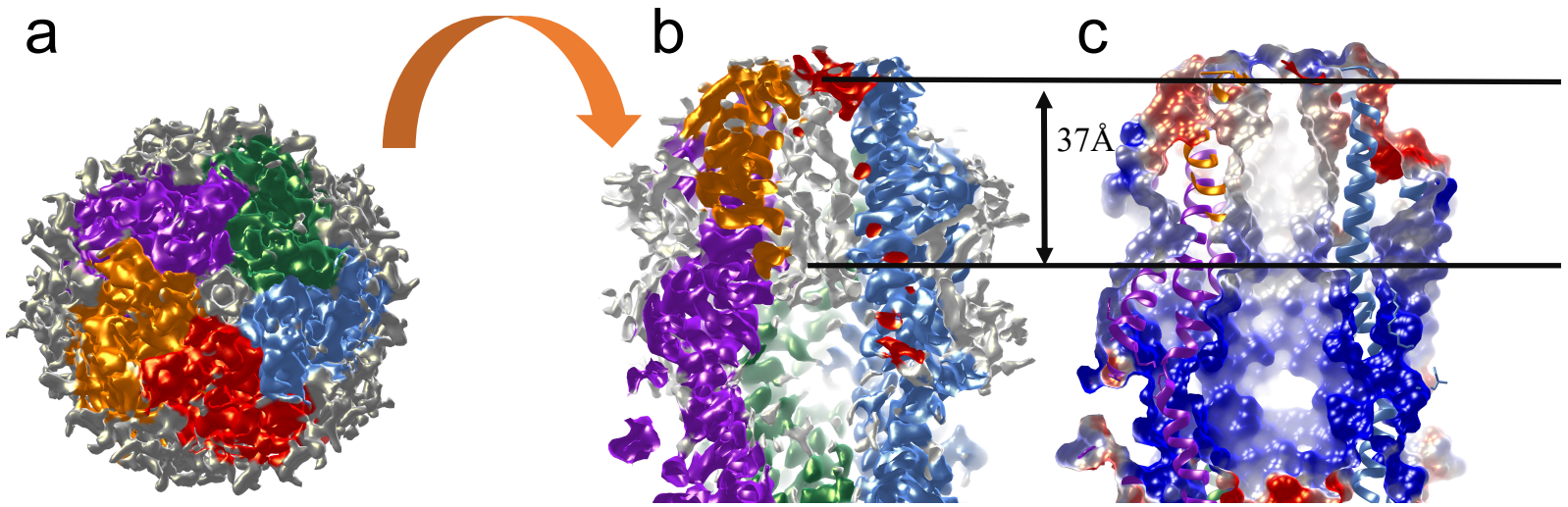


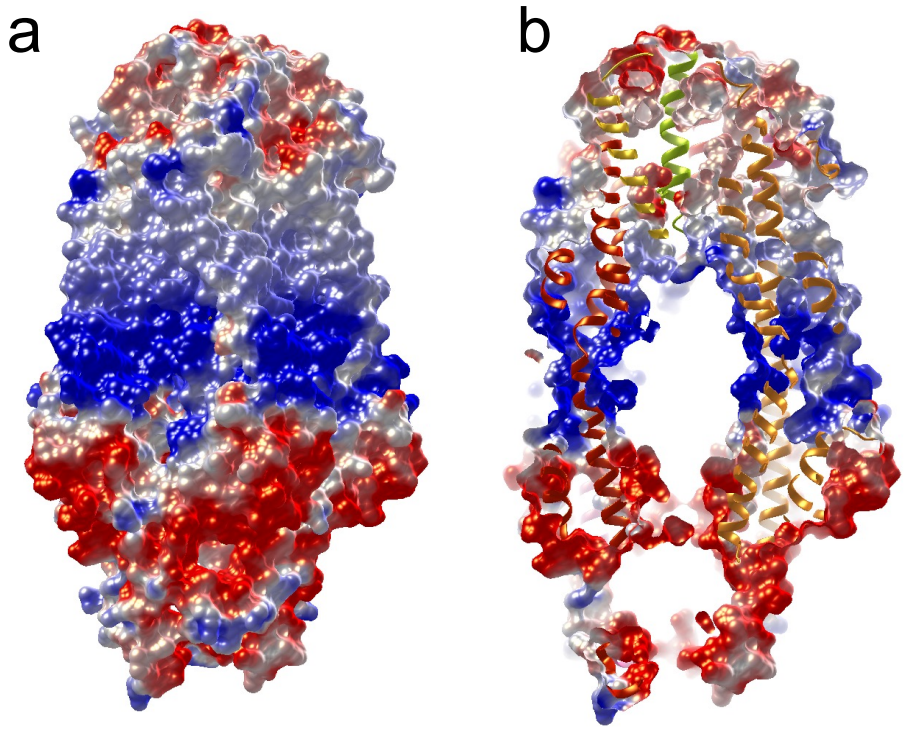
Supplementary Figure S7: uncropped SDS-PAGE and western blot corresponding to a) Figure 5b and b) Figure 5c.

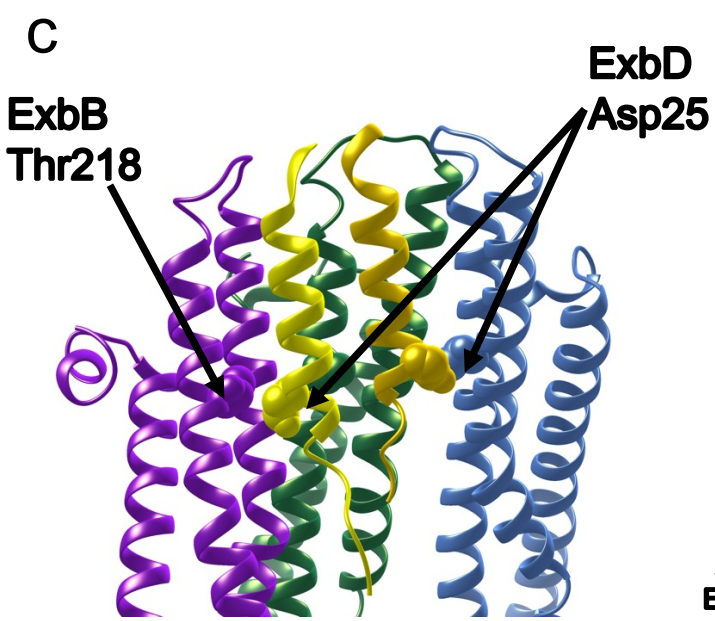


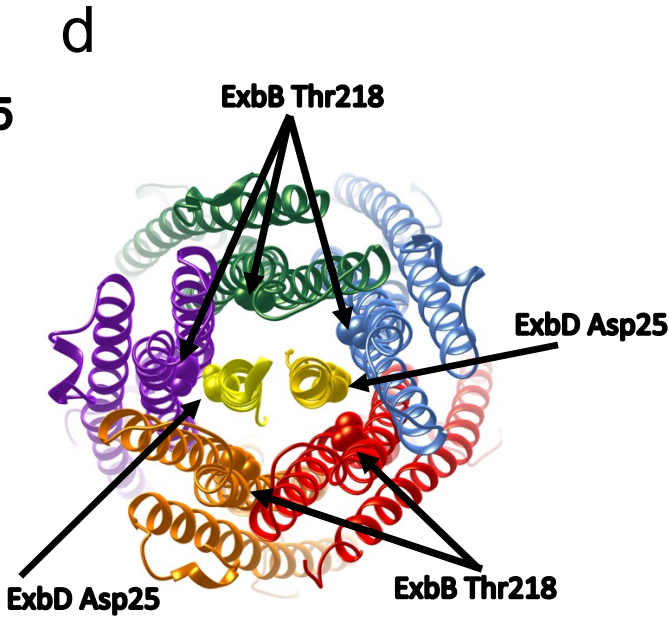
Supplementary Figure S8: sequence conservation in ExbB. structure of ExbB with one monomer ramp-colored with respect to sequence conservation using sequences retrieved in Table SI and Consurf server. Worm diameter also increases with conservation. In the transmembrane region the TM1 is the least conserved.

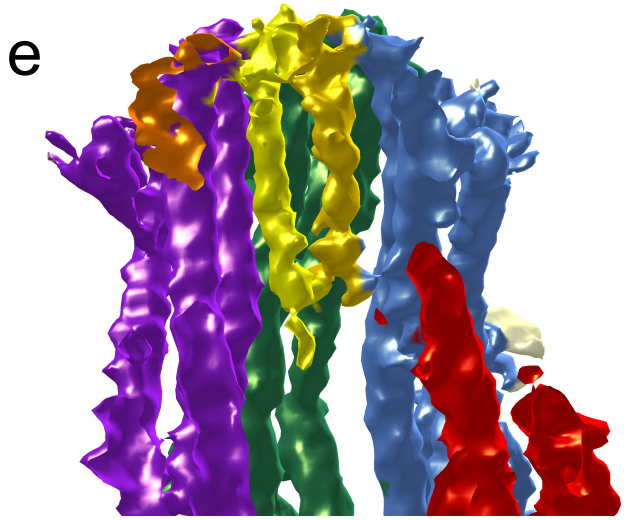


Supplementary figure S9: ExbB pentamer hosts density inside its transmembrane channel. a and b, top and side views of the ExbB density colored with respect to protein chains as in Figure 8. the grey regions show extra density not accounted for by the model. c, electrostatic surface inside the channel corresponding to b. The two black lines show the cytoplasmic and periplasmic limits of the channel density. They are 37Å away from each other.

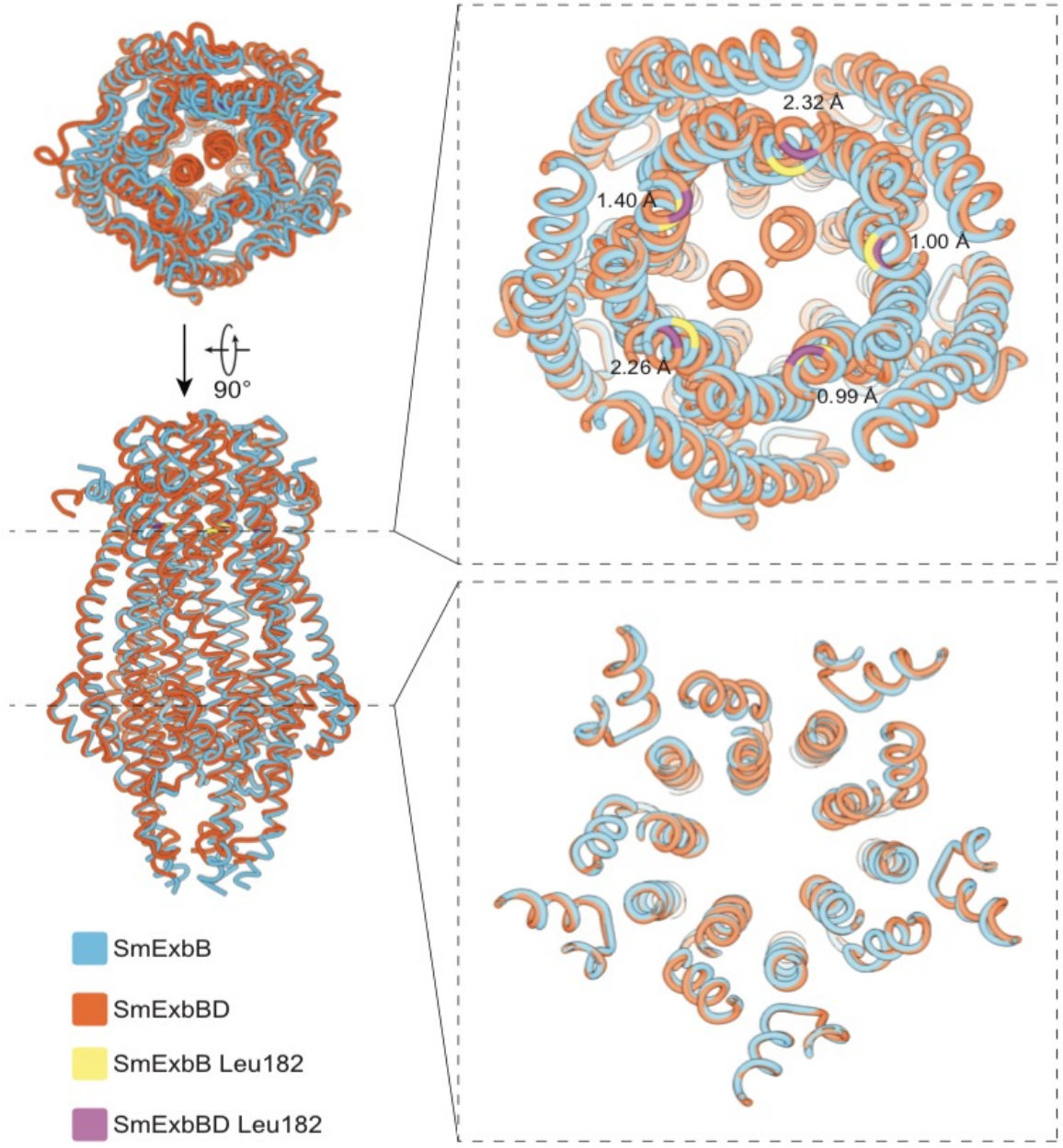








Supplementary Figure S10: electrostatic surface and relative orientation of conserved residues in the complex core. Potential surface of $ExbBD_{Sm}$ determined with the APBS plugin (a, external; b, sagittal section) Respective positions of the conserved Asp 25 residues from both ExbD TM helices and of the conserved Thr 218 residues from ExbB TM2 helices. c, sagittal section across the pentamer; d, view from the top. e, same view as c with the density map.



Supplementary Figure S11. Comparison of ExbBSm with ExbBDSm (top inset: section view from the periplasmic side, bottom inset: section view from the cytoplasmic side): both ExbB structures are represented in cartoon (ExbB, lightblue colour, ExbBD lightbrown colour, respectively). A specific Leu 182 residue at the periplasmic entrance is highlighted with the Cα distances between the two molecules.

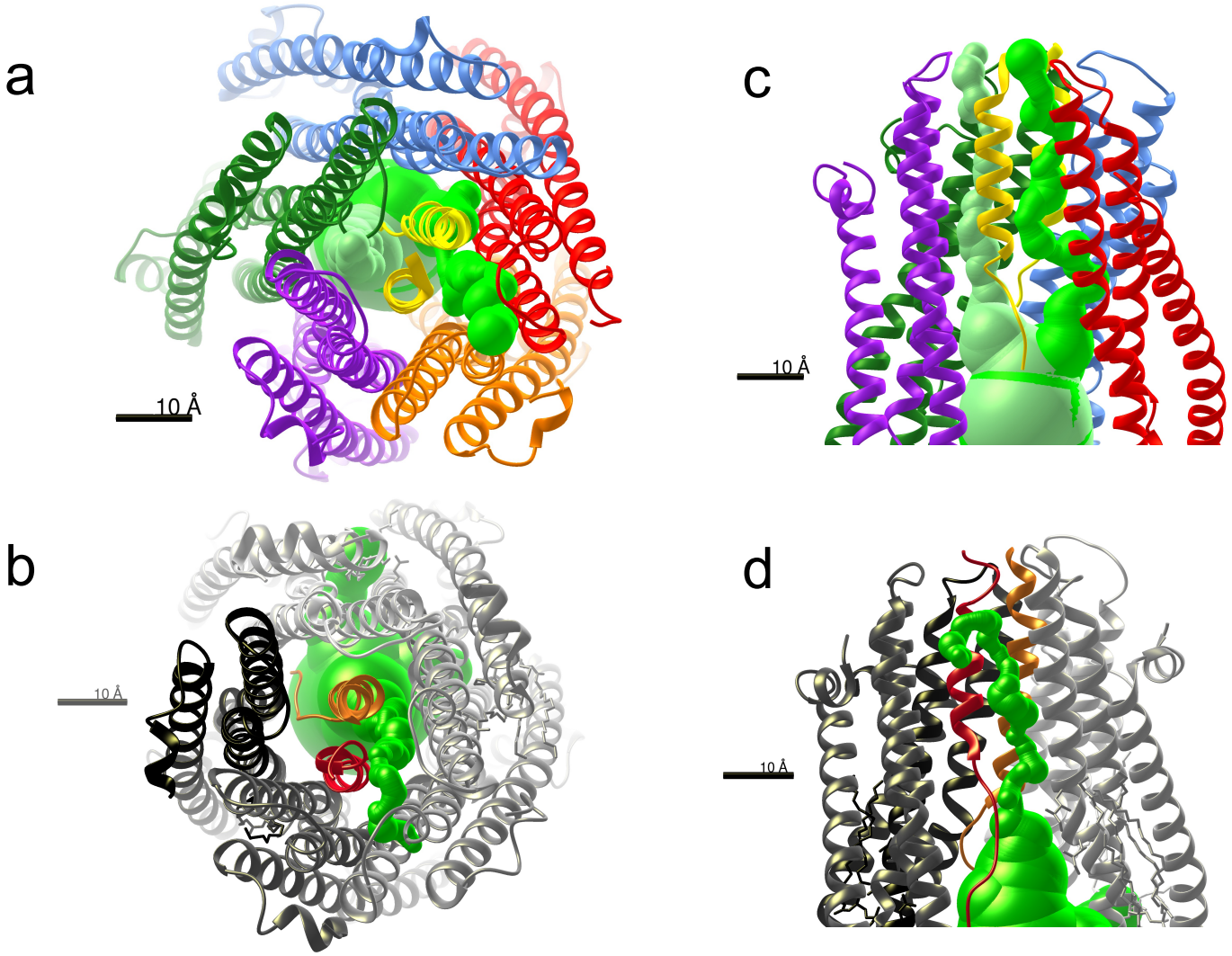
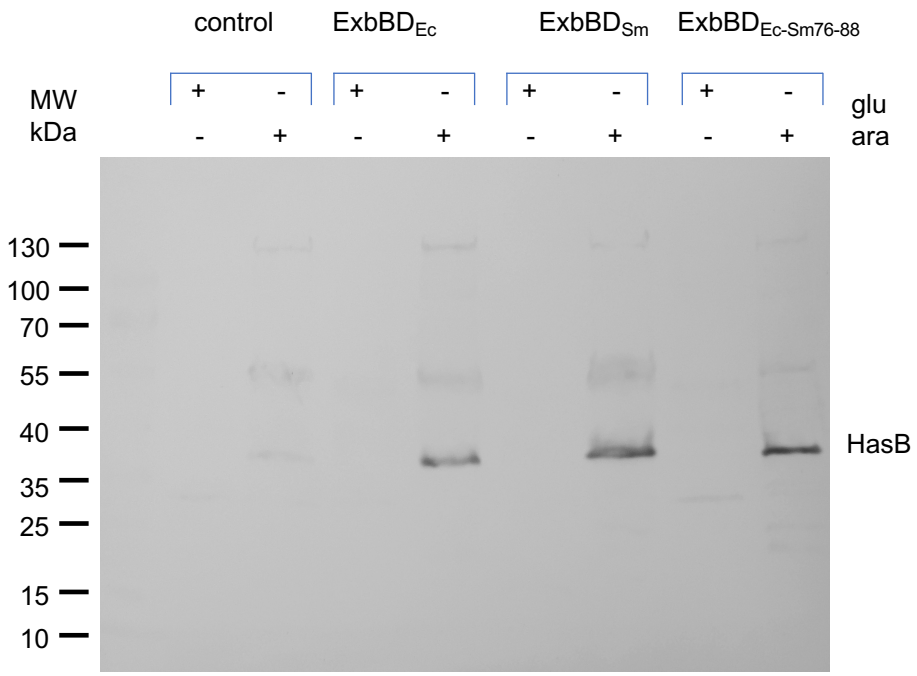
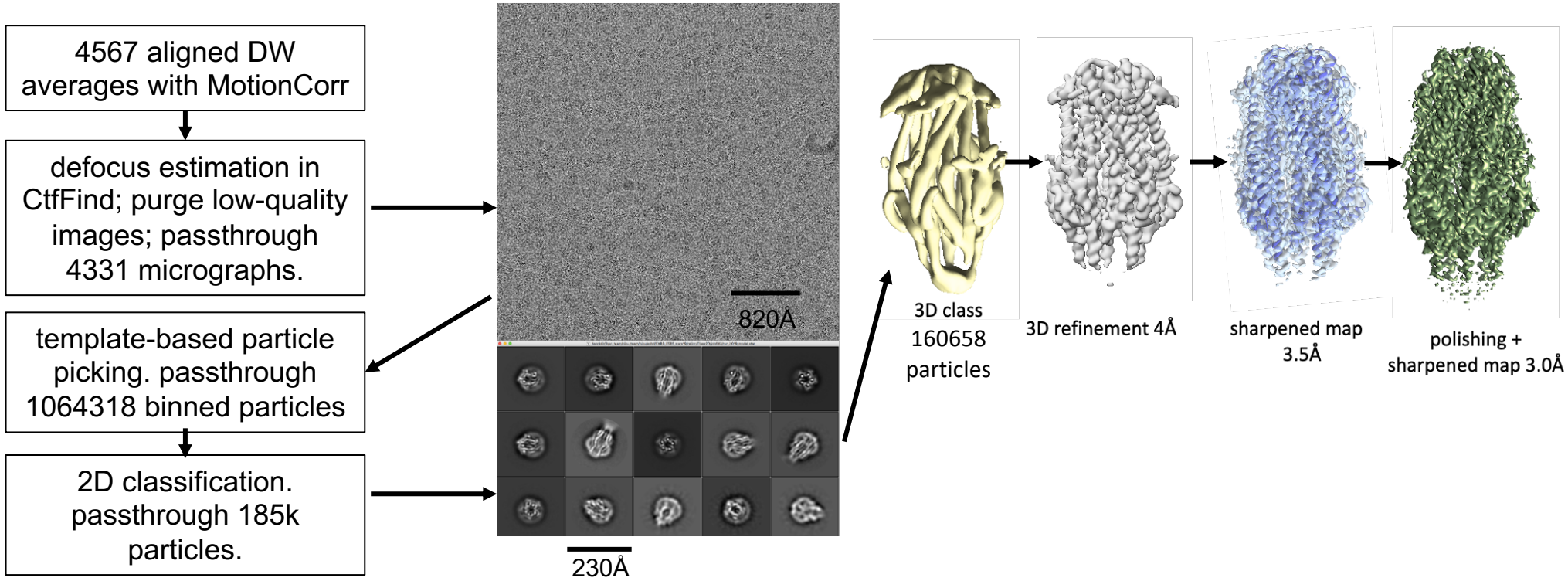


Figure S12: Visualisation of “tunnels”. ExbBD structures from *S. marcescens* (a and c) and *E. coli* 6TYI structure (b and d), as calculated by the MoleOnline server with the same parameters. The Sm complex is colored as in Figure 8 and the Ec complex is coloured in shades of grey for ExbB and orange and red for ExbD. The tunnels are the green volumes running through the structure, viewed from the periplasmic space (a and b), and from the side (c and d). The average tunnel diameter is 3Å for *S. marcescens* and 2Å for *E. coli.*



Supplementary Figure S13. ExbBD co-expression is necessary for HasB stabilisation. Immunodetection of HasB in whole cells of *E. coli* JP313Δ*exbBD*Δ*tonB*(pHasB33) also harbouring pBAD24 (control), pBAD24ExbBD$_{Ec}$, pBAD24ExbBD$_{Sm}$ or pBAD24ExbBD$_{Ec-Sm76-88}$, in the presence of either glucose (1mg/ml) or arabinose (40µg/ml), indicated by the + signs. The equivalent of 0.2OD$_{600nm}$ was loaded in each lane. The molecular weight markers were located using a more saturated version of this image and are figured by lanes.

a



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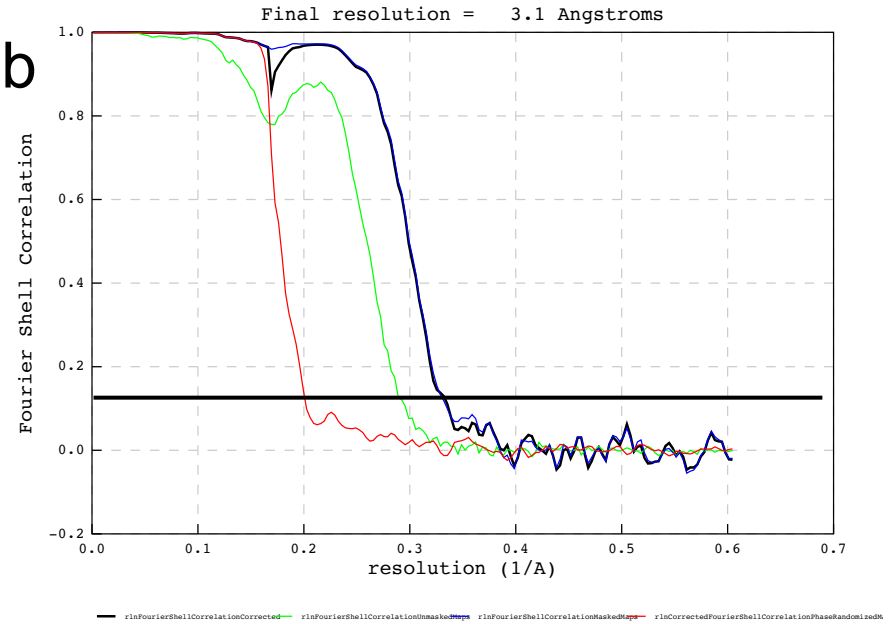


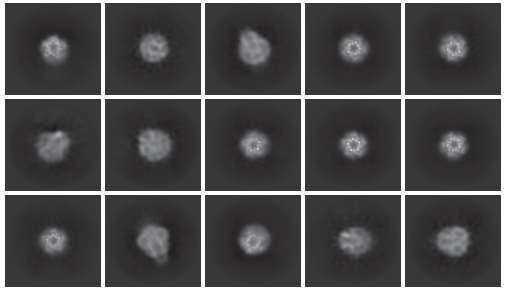
Supplementary Figure S14: Processing of ExbB cryo-EM data set (a) and final Fourier Shell Correlation plot (b).

4043 aligned DW averages

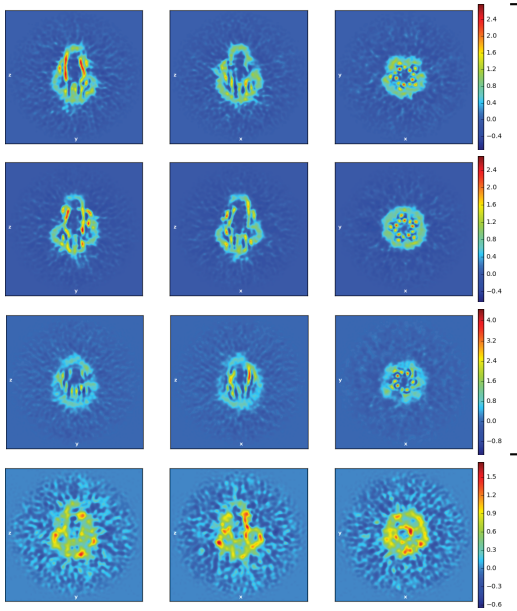
Defocus estimation in gCTF
Purging low quality images
Passthrough: 3028 images

Template based particle picking and prunning based on NCC and power score
Passthrough: 1 373 k particles

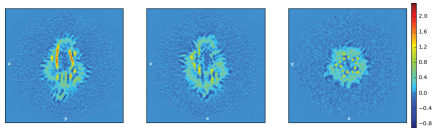
2D classification
Passthrough: 609 k particles

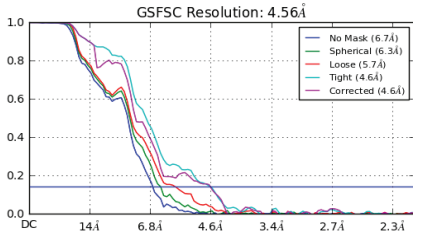


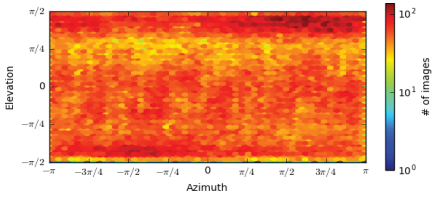
2 Cycles of Ab Initio model generation and 3D classification
Passthrough:
180 k particles (cycle1)
158 k particles (cycle2)

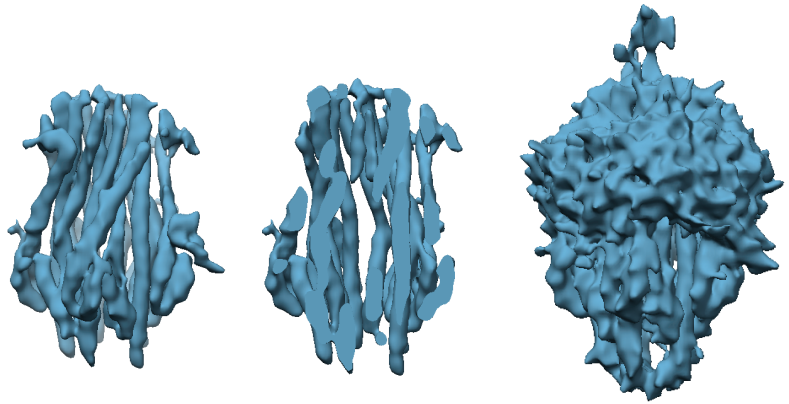


Non Uniform Refinement in C1

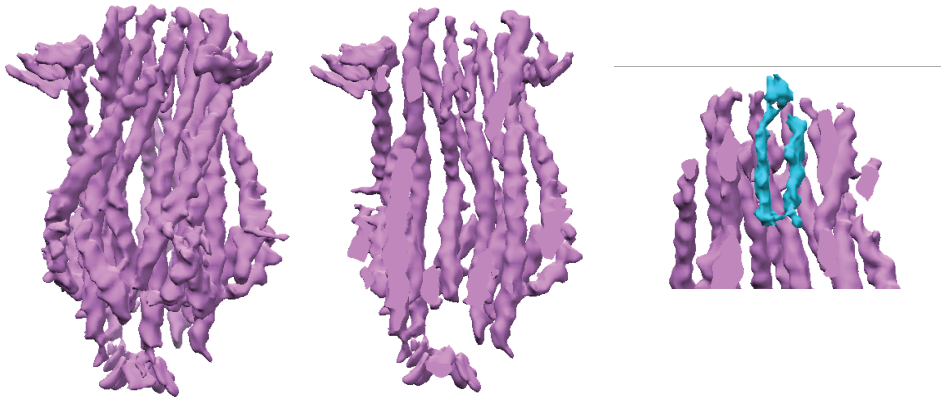


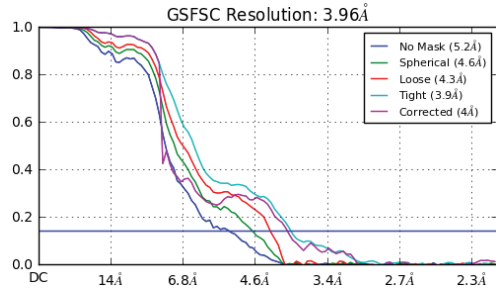






Signal subtraction of the detergent micelle in the concensus model
Localized refinement





Supplementary Figure S15: Processing of ExbB-D cryo-EM data set

Afipia broomeae
Agrobacterium rhizogenes
Aliihoeflea sp.
Aminobacter aminovorans
Amorphus coralli
Ancylobacter aquaticus
Aquamicrobium aerolatum
Aurantimonas manganoxydans
Aureimonas flava
Azorhizobium caulinodans
Azospirillum brasilense
bacterium A52C2
Bartonella apis
Beijerinckiaceae bacterium
Blastochloris sp.
Bosea vaviloviae
Bradyrhizobium yuanmingense
Brucella sp.
Chelatococcus asaccharovorans
Ciceribacter lividus
Devosia psychrophila
Dongia mobilis
Enterovirga rhinocerotis
Erythrobacter
Falsochrobactrum ovis
Geminicoccus roseus
Granulibacter bethesdensis
Haematobacter massiliensis
Hansschlegelia zhihuaiae
Hartmannibacter diazotrophicus
Hoeflea olei
Hyphomicrobium nitrativorans
Insolitispirillum peregrinum
Kaistia granuli
Ketogulonicigenium robustum
Labrys okinawensis
Magnetospirillum gryphiswaldense
Mangrovicella endophytica
Mesorhizobium loti
Methylobrevis pamukkalensis
Methylocapsa acidiphila
Methyloceanibacter stevinii
Methylocella silvestris
Methyloligella halotolerans
Methylopila sp.
Methylovirgula ligni
Microvirga aerophila
Neorhizobium alkalisoli
Nitratireductor pacificus
Nitrobacter hamburgensis
Nitrospirillum amazonense
Niveispirillum cyanobacteriorum
Ochrobactrum thiophenivorans
Paenirhodobacter enshiensis
Paracoccus aminovorans
Paramesorhizobium deserti
Parvibaculum lavamentivorans
Phaeospirillum fulvum
Phreatobacter stygius
Phyllobacterium zundukense
Proteobacteria bacterium
Pseudaminobacter manganicus
Pseudochrobactrum asaccharolyticum
Pseudolabrys taiwanensis
Pseudorhizobium pelagicum
Pseudorhodoplanes sinuspersici
Reyranella massiliensis
Rhizobium tropici
Rhodoligotrophos sp.
Rhodomicrobium udaipurense
Rhodopseudomonas pseudopalustris
Rhodospirillum centenum
Rhodovarius sp.
Roseococcus sp.
Roseospirillum parvum
Sinirhodobacter sp.
Sinorhizobium sp.
Skermanella aerolata
Sphingobium chlorophenolicum
Sphingomonas sp.
Sphingopyxis sp.
Sphingorhabdus sp.
Starkeya novella
Tardiphaga robiniae
Telmatospirillum siberiense
Variibacter gotjawalensis
Xanthobacter autotrophicus

Table S1: a list of selected species of Alphaproteobacteria where “extralong” ExbB’s are found. All those species also contain HasB orthologs, except for the ones in red.

Strains :

E. coli K12 C600

E. coli K12 C600*ΔhemA::Km*^{r}*ΔexbBD*

E. coli K12 C600*ΔhemA::Km*^{r}*ΔexbBDΔtonB*

E. coli K12 XL1-Blue

E. coli K12 JP313

E. coli K12 JP313*ΔexbBDΔtonB*

E. coli K12 C600*ΔexbBD*

E. coli BL21DE3

S. marcescens Db11

Plasmids :

pBAD24: lab collection

pBAD33: lab collection

pAM238: lab collection

pAMHasISRADE: lab collection

pAMHasISRADEB: this work

pBADExbBD$_{Ec}$: this work

pBADExbBD$_{Sm}$: this work

pBADExbBD$_{Ec\text{-}Sm76\text{-}88}$: this work

pBADExbBD$_{Ec\text{-}Sm76\text{-}84}$: this work

pBADExbBD$_{Sm\text{-}Ec39\text{-}51}$: this work

pBADExbBD$_{delextss}$: this work

pBADHasB: lab collection

pBADExbBD$_{Sm}$His6: this work

pBADExbB$_{Sm}$His6: this work

pBADExbB$_{Smdelextss}$His6: this work

Oligonucleotides:

ExbBDSm5′	5′-GGAGGAATTCACCATGAAAACGGCTGGCAAGAAT -3′
ExbBDSm3′	5′-AAGCTTGCATGCCTATTTGGCGGCGCCTTCCA-3′
SphIHisCtexbDSm	5′-TTGCATGCCTAATGGTGATGGTGATGGTGTTTGGCGCGCCTTC-3′
BglIIexbDSm	5′-GCGCTTAAATGAAGATCTGGACGACAGCGG-3′
ExbBD5c	5′-CAGGAGGAATTCACCATGGGTAATAATTTAATGCAGACGGA-3′
ExbBD3c	5′-AAGCTTGCATGCTTACTTCGCTTTGGCGGTTTCTT-3′
PBADFOR	5′-CTGACGCTTTTTATCGCAAC-3′
ExbBHis6	5′-AAGCTTGCATGCCTAATGGTGATGGTGATGGTGCCCCGCCCGCAGTTG-3′
ExbBEcSm76-88.1	5′P-GGCAGTGAACTGCTGCGCGCCAAGCGTCGCCTTAAGCGCGAG-3′
ExbBEcSm76-88.2	5′P-TTTAGCGAACAAAATGGTCCAGGTGACTACGGAGGCCAAAAT-3′
ExbBEcSm76-84.1	5′P-CTAAAGGCAGTGAATTCTTCAATCAGAAGCGTCG-3′
ExbBEcSm76-84.2	5′P-CGAACAAAATGGTCCAGGTGACTACGGAGGCCA-3′
ExbBSmEc39-51.1	5′P-GCGTAGAGTTCTTCAATCAGAAGCGCCGTCTGCGTCGCGA-3′
ExbBSmEc39-51.2	5′P-TCTTACTGAAGAAGATTGCCCAGGTCACGATAGACGCCAG-3′
ExbBdelextss1	5′P- GAAACCCGCGGCATGGACCTGTCCATTTGGGG-3′
ExbBdelextss2	5′P- TGCCTGCGCGCTGCCGGCCAGCCCCACAA-3′

Table S2: list of strains, plasmids and oligonucleotide sequences used in this work