

Coordinated Rearrangements between Cytoplasmic and Periplasmic Domains of the Membrane Protein Complex ExbB-ExbD of *Escherichia coli*

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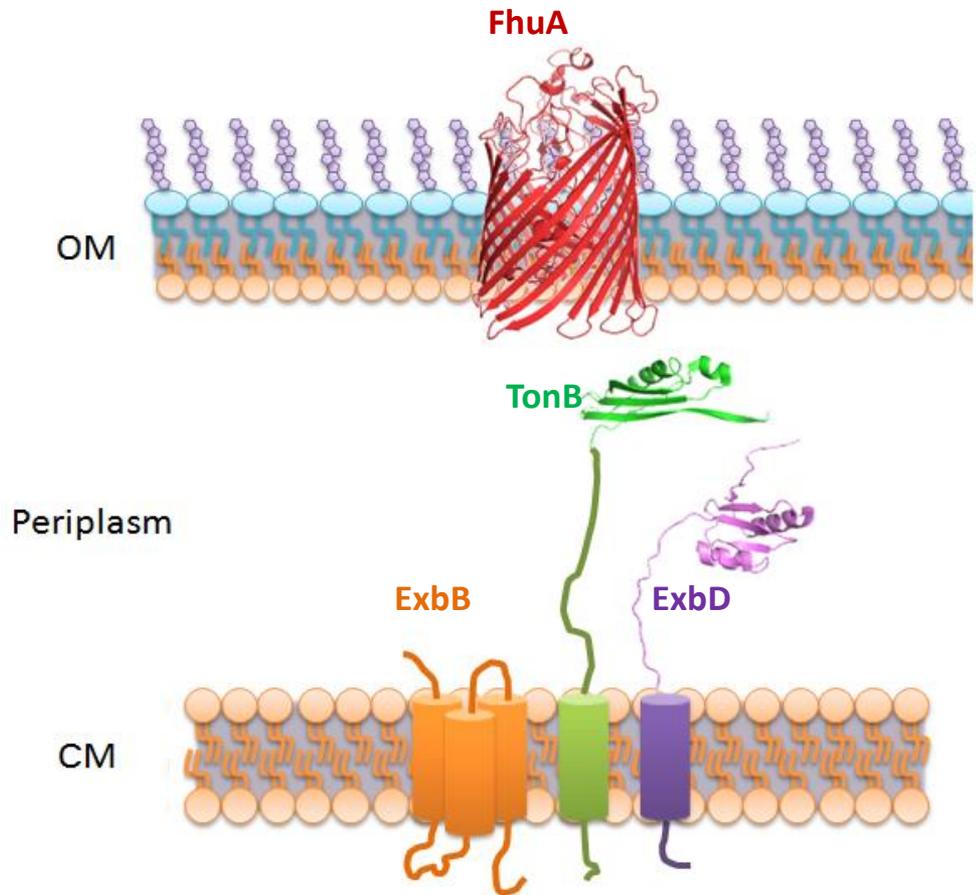
Structure: 2014 May 6;22(5):791-7

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Introduction

ExbB composed of **three** **transmembrane** (TM) helices and can form **dimers** and **tetramers** (Higgs *et al.*, 1998).

ExbD composed of **one TM helix**. It can form **homodimers** as well as **heterodimers** with TonB (Ollis *et al.*, 2009).

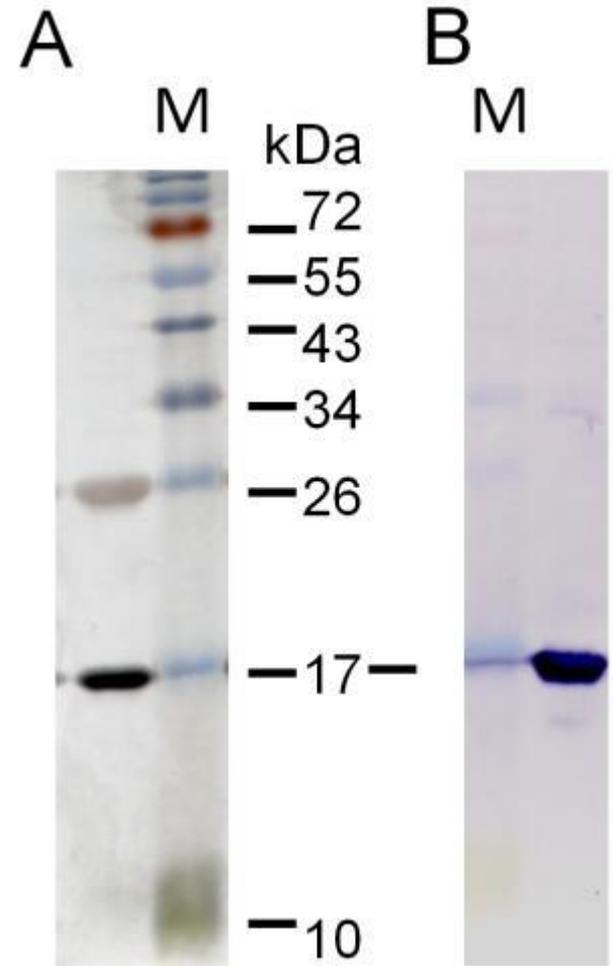


Adapted from (Ivanov, 2012)

Purification and Biochemical Characterization of ExbB–ExbD-His⁶

(A) IMAC-eluted proteins were detected by SDS-PAGE and silver staining. ExbB (26 kDa) coeluted with ExbD-His⁶ (17 kDa).

(B) Anti-His⁶ Western blot identifying ExbD as the sole His-tagged protein.

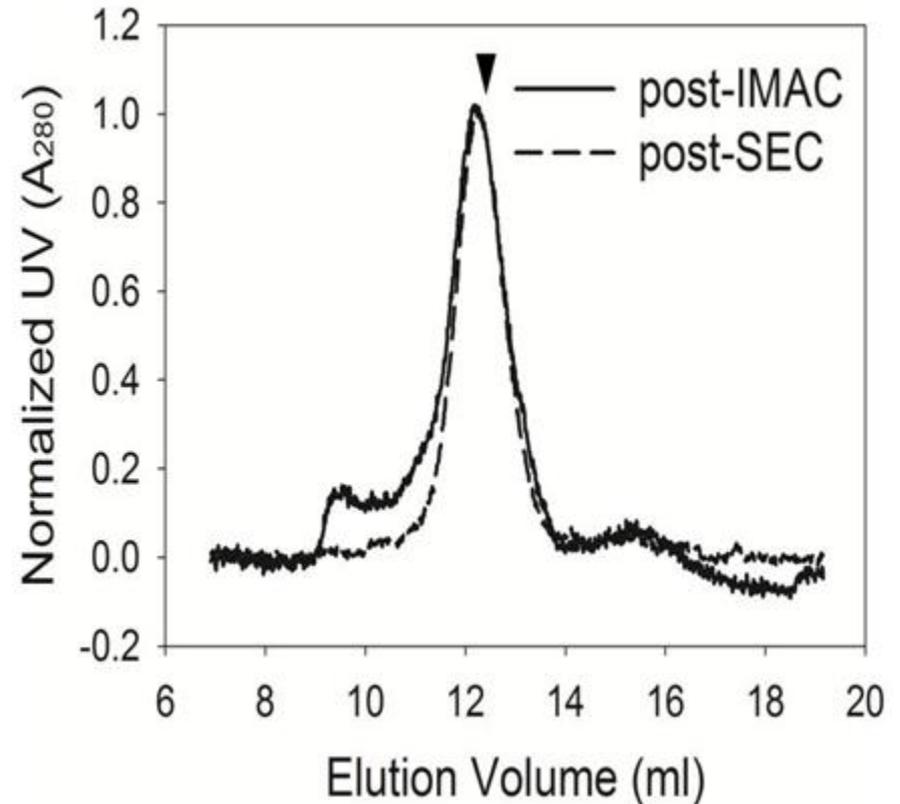


Size Exclusion Chromatography of IMAC-Eluted Proteins

A single fraction from
preparative SEC was
monodisperse

DDM was quantified by
1D NMR

EXbB-ExbD content =
 $60.5\% \pm 5.5\%$



Molecular Mass of the Principal Protein-Detergent Complex

A- BN-PAGE of ExbB–ExbD-His⁶

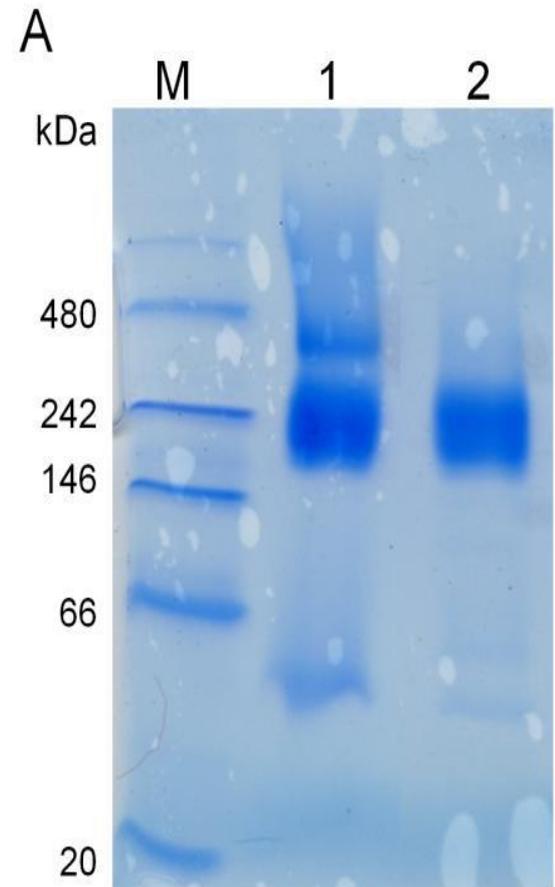
post-IMAC (lane 1)

post-SEC (lane 2)

The principal PDC migrated to
~242 kDa.

B- Multiangle laser light scattering

PDC ~237 ± 5 kDa



The Molar Ratio of ExbB and ExbD in the Principal Protein-Detergent Complex

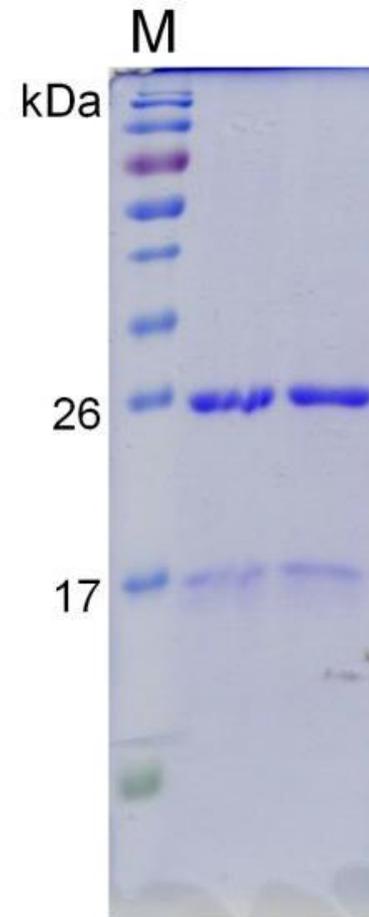
Quantitative Coomassie staining of the protein constituents separated by SDS-PAGE revealed a molar ratio of

2 ExbB: 1 ExbD

EXbB-ExbD content = $60.5\% \pm 5.5\%$

4 ExbB and 2 ExbD molecules: 138.6 kDa

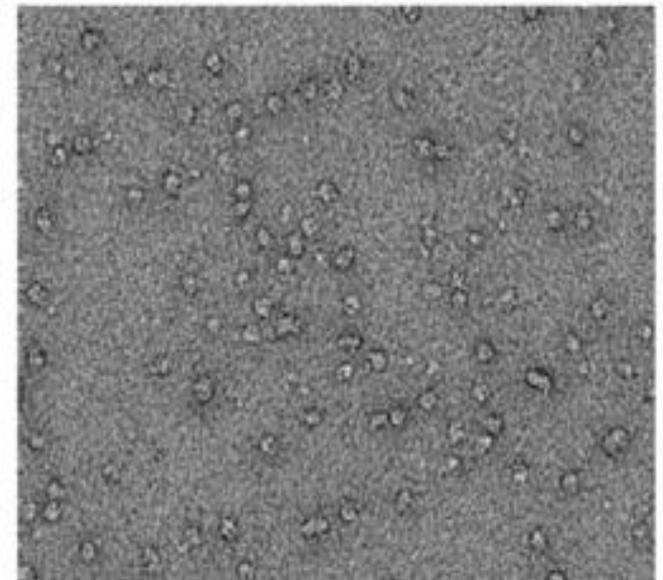
plus **~100 kDa** DDM.



Single Particle Electron Microscopy of ExbB–ExbD-His⁶

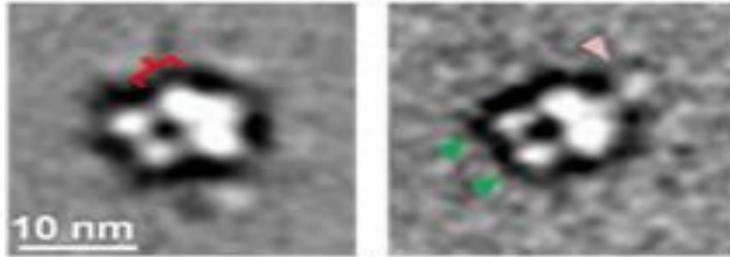
ExbB4–ExbD2-His⁶ negative
staining with uranyl formate

homogeneous distribution of
particles approximately **10 nm in
diameter** and present in **various
orientations** on the EM grid

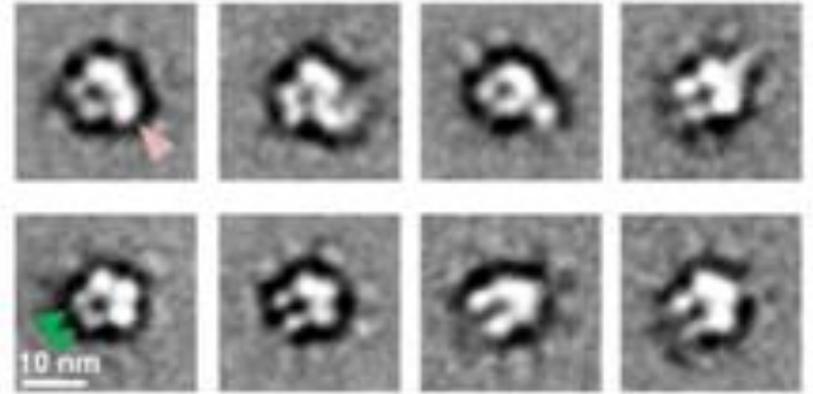


Single Particle Electron Microscopy of ExbB–ExbD-His⁶

A



B



28,000 images were validated with the ISAC and clustered into stable classes (449 classes)

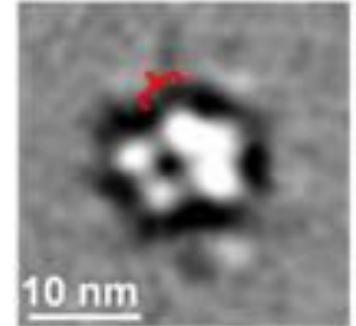
1- a predominant density “4x10 nm” (**red bracket**)

2- The latter density (**Pink arrowhead**)

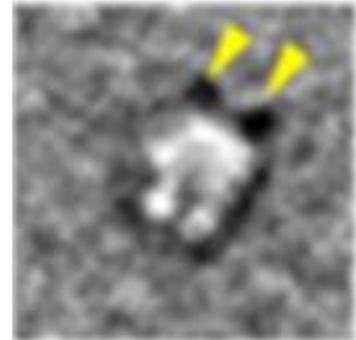
3- Two density extensions below the central density (**green arrowheads**)

Identification of the TM Domains, Periplasmic and Cytoplasmic Sides of the ExbB–ExbD-His⁶ Complex

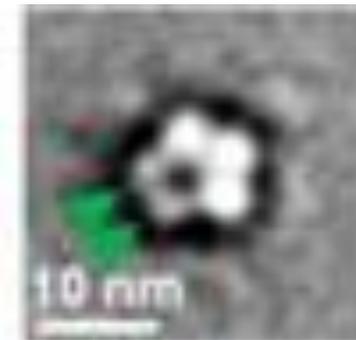
A- TM domains: 14 TM domains (Central density)



B- Periplasmic sides: Lateral density



C- Cytoplasmic sides: Densities below the TM region



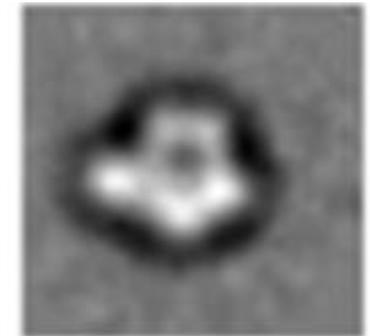
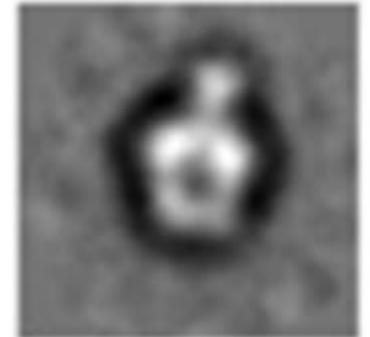
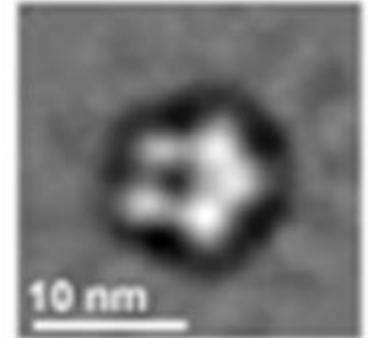
2D images of ExbB–ExbD-His⁶

The ISAC classes were averaged based on the ExbD periplasmic position:

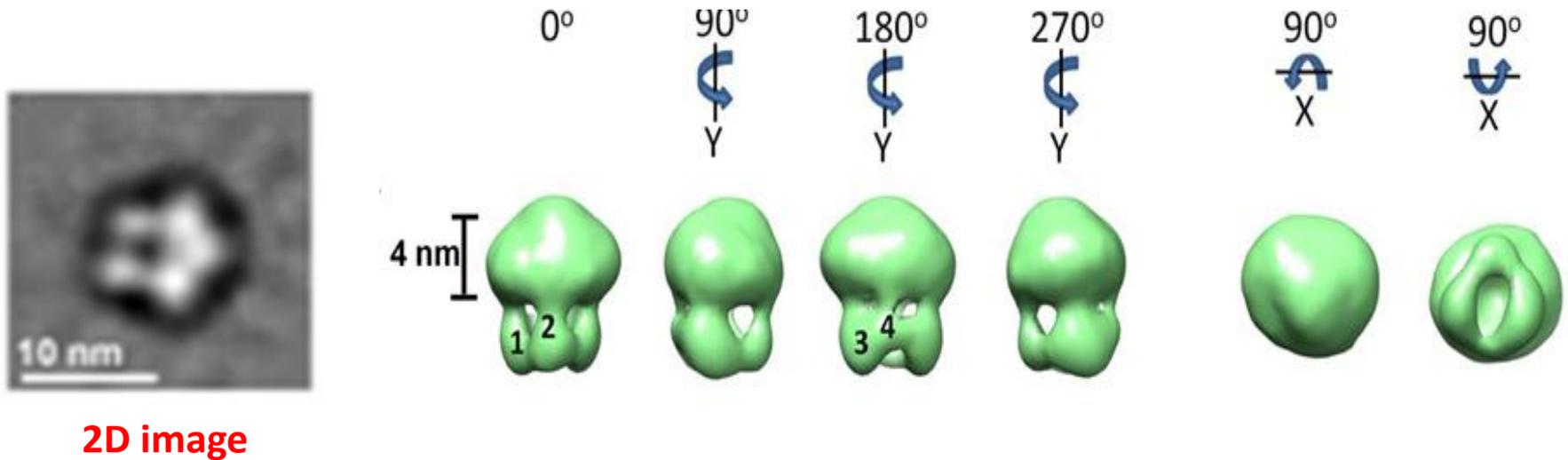
1- ExbD-undefined state

2- ExbD-extended state

3- ExbD-membrane-parallel state



3D EM Maps of the ExbD-Undefined State

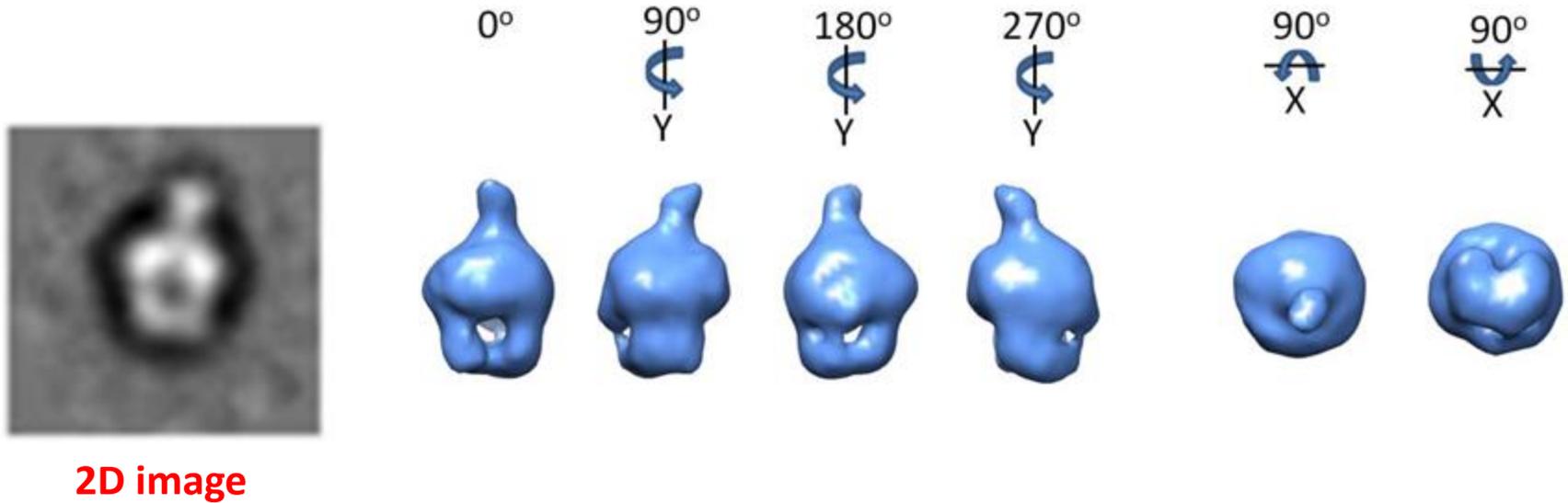


ExbD-undefined state shows:

** four densities (labeled) extending from the TM region and forming a ring when viewed from the cytoplasmic face.

** No density above the TM domain was observed.

3D EM Maps of the ExbD-Extended State

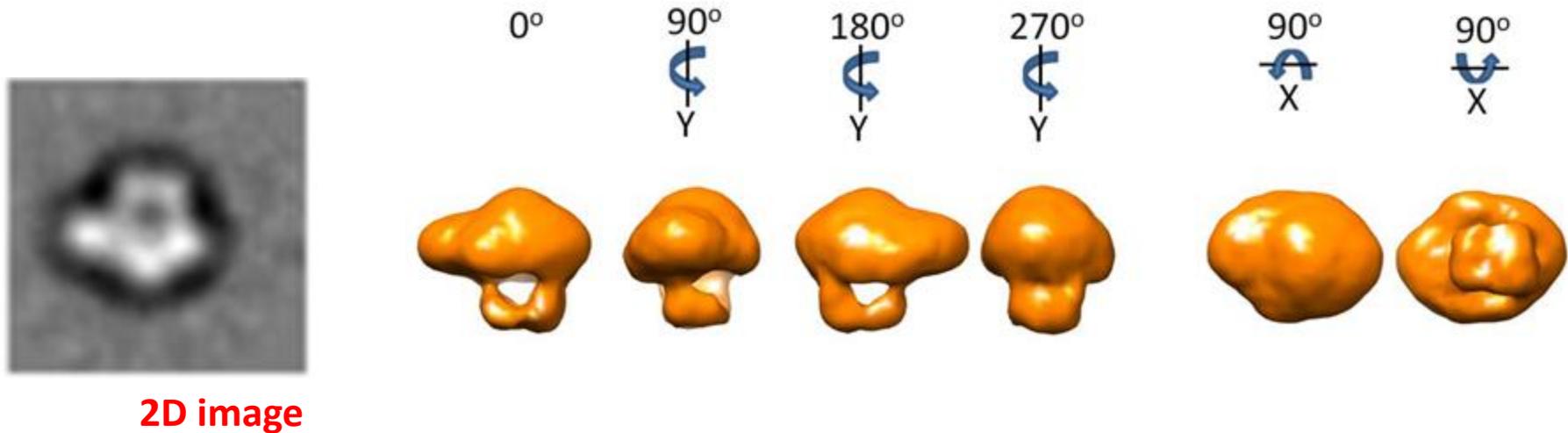


The ExbD-extended state shows:

****Two thick densities below the TM region.**

****They form a compact arrangement as viewed from the cytoplasmic face.**

3D EM Maps of the ExbD-Membrane-Parallel State

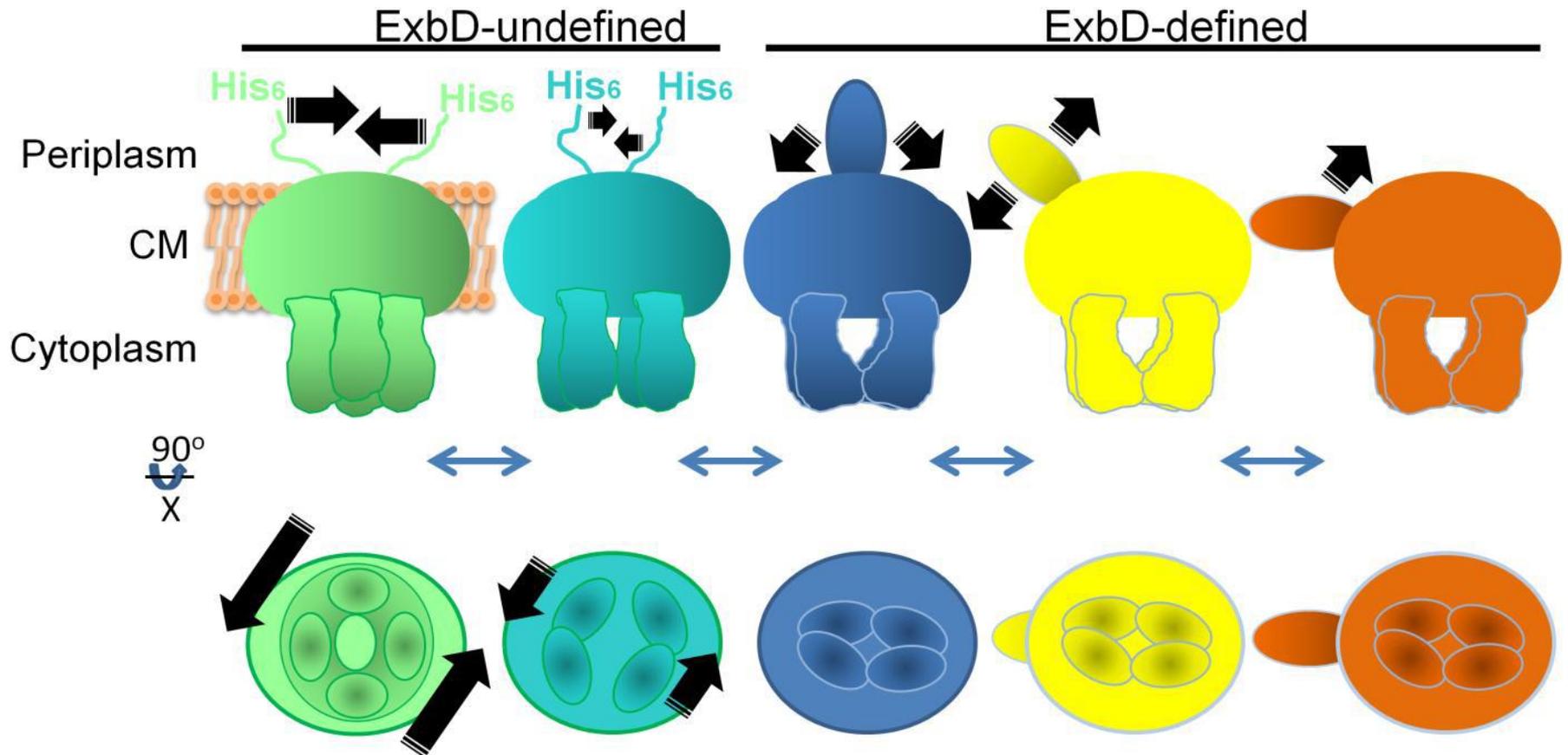


The ExbD-membrane-parallel state shows:

**Two thick extensions below the TM region

** They form a compact arrangement on the cytoplasmic side of the membrane protein complex

Conclusion



Schematic Representations of ExbB–ExbD–His⁶



Acknowledgment

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