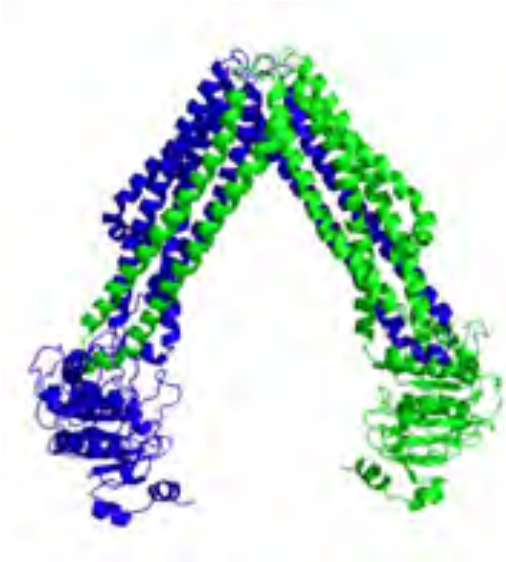
Cryo-EM studies of Membrane proteins in Peptidiscs & Nanodiscs

Tom Walz
The Rockefeller University

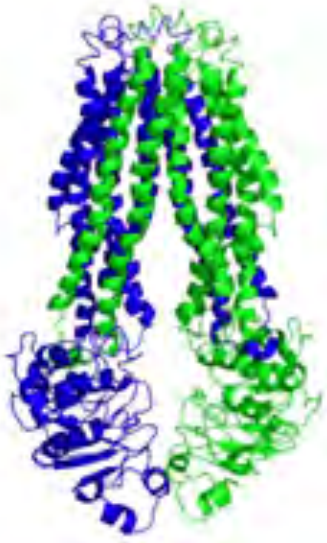
International SMALP Conference

New York, March 2020

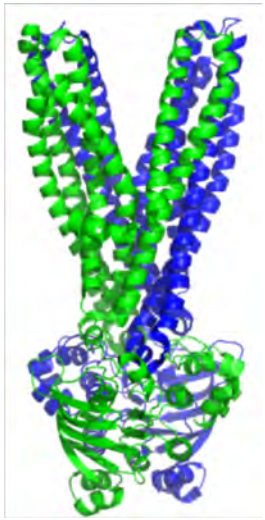
Crystal structures of ABC transporter MsbA in detergent



E. coli
5.3 Å
Inward wide open
No nucleotide



V. cholerae
5.5 Å
Inward twisted open
No nucleotide



S. typhimurium
3.7 Å
Outward open
AMPPNP

Are all conformations meaningful ?

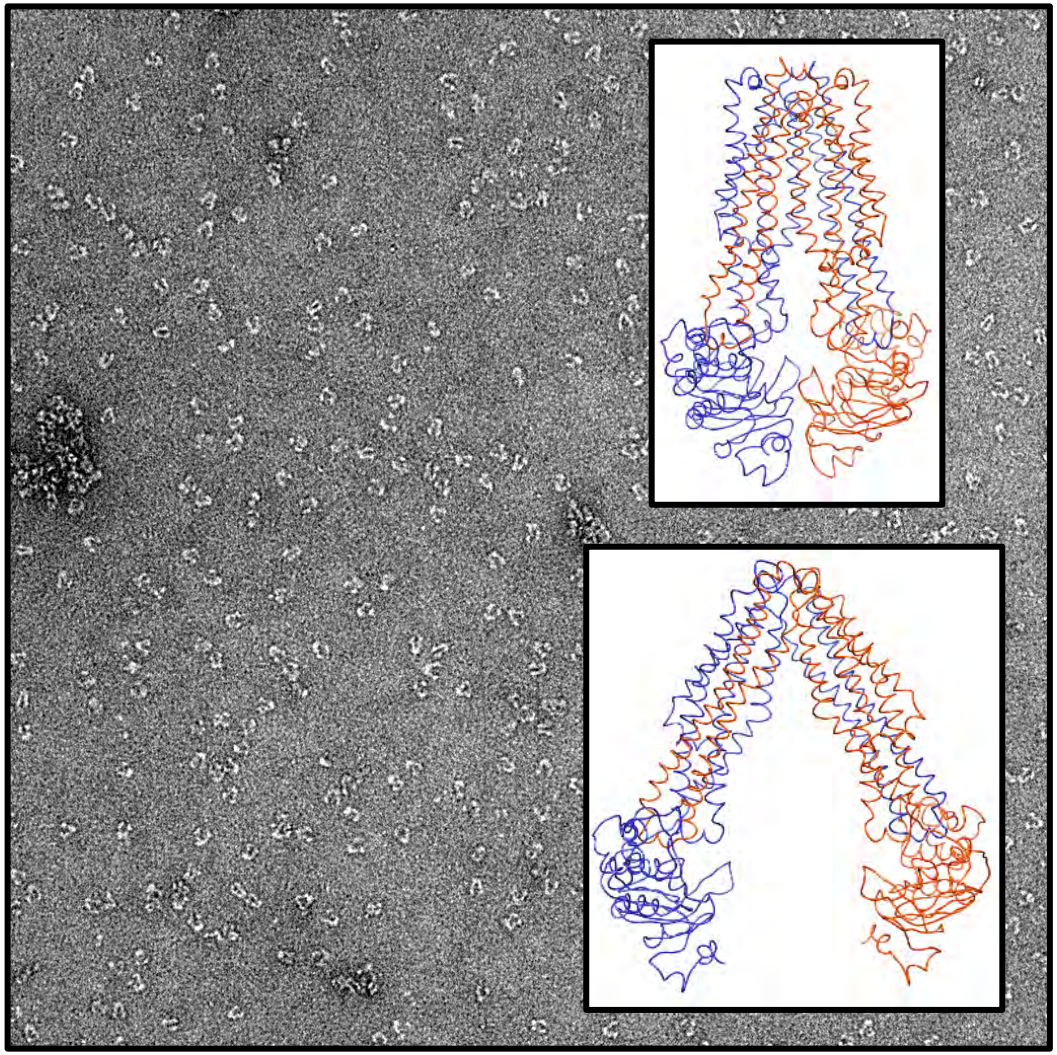
Does the lipid bilayer have an effect ?

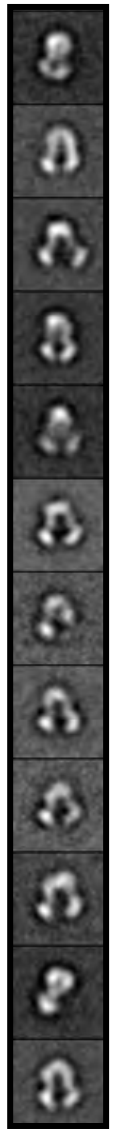
How does MsbA specifically recognize LPS ?

How does MsbA flip LPS ?

Ward *et al.* (2018) *Proc. Natl. Acad. Sci. USA* 104: 19005-19010

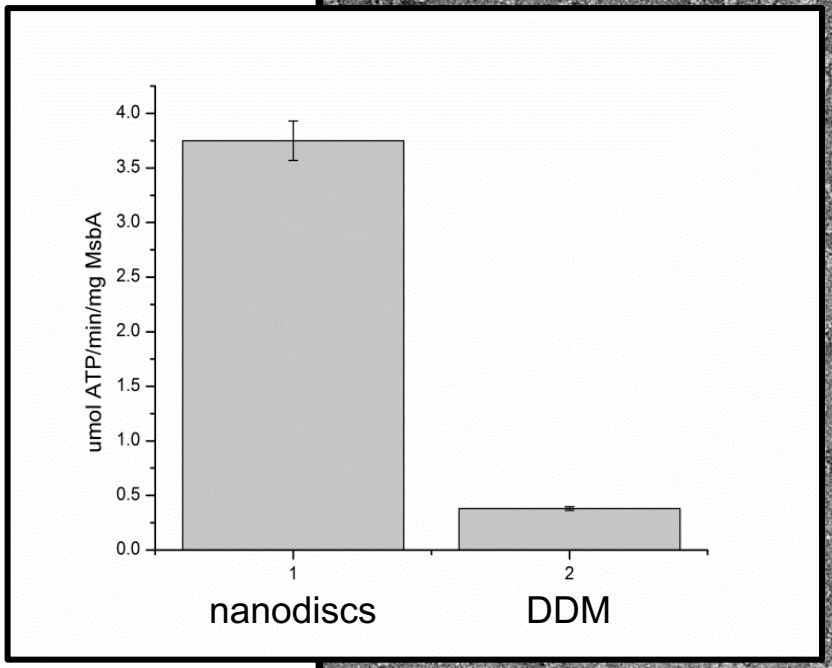
Nucleotide-free MsbA in detergent (dodecyl maltoside)

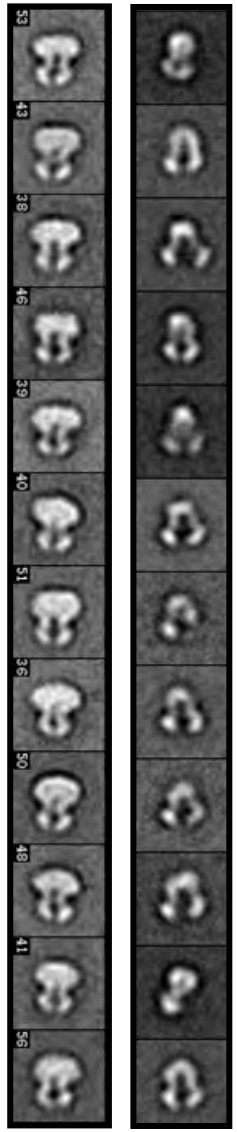




Mi *et al.* (2017) *Nature* 549: 233-237

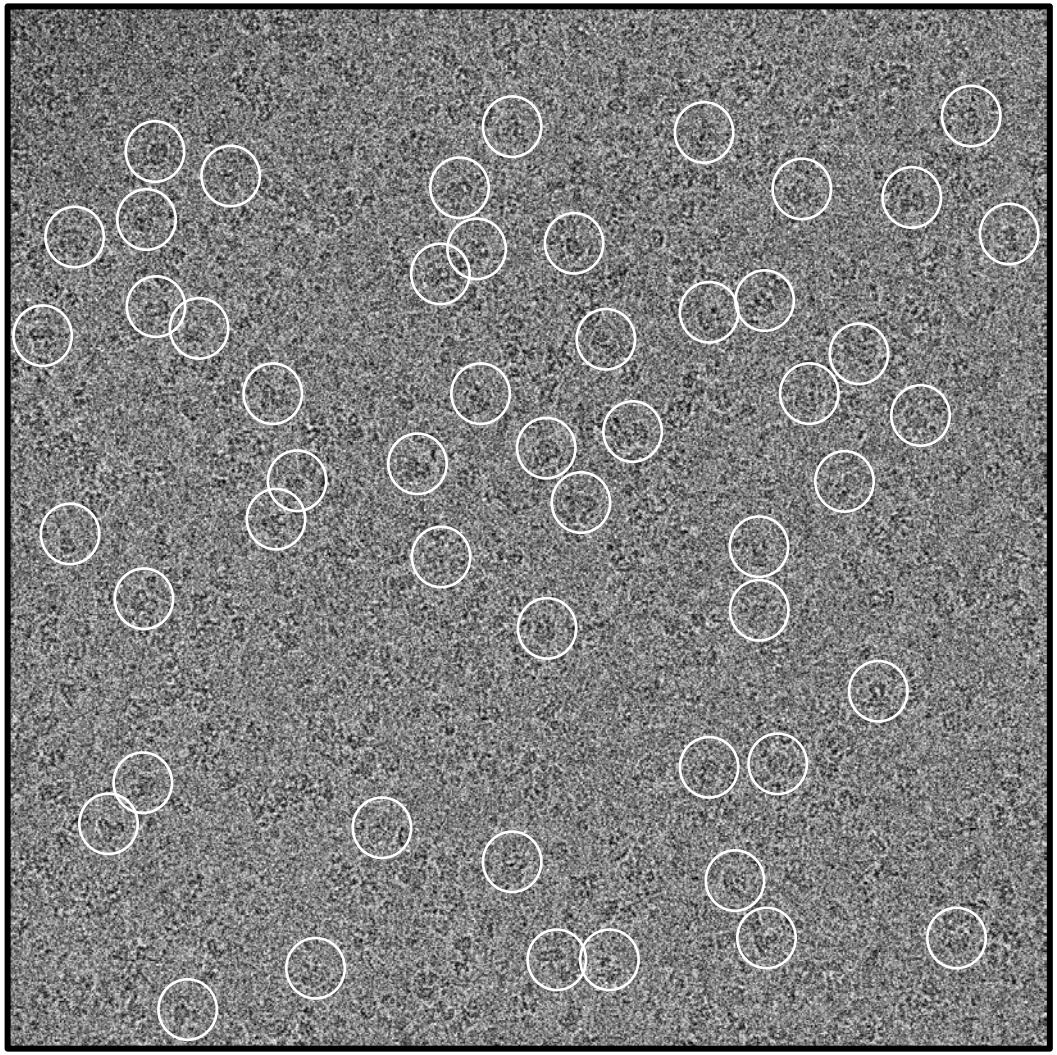
Nucleotide-free MsbA in nanodiscs (MSP1D1 & *E. coli* polar lipids)

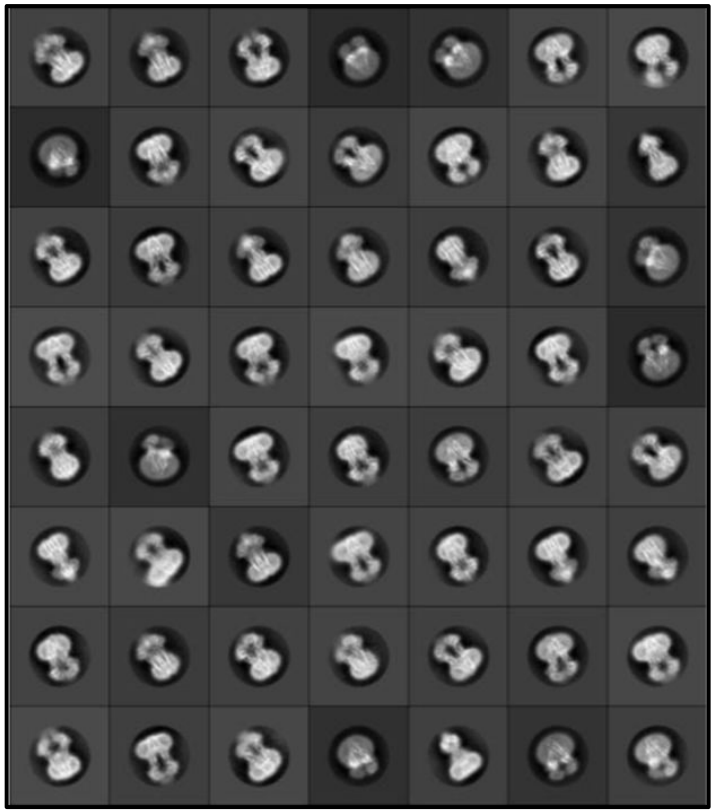




Mi *et al.* (2017) *Nature* 549: 233-237

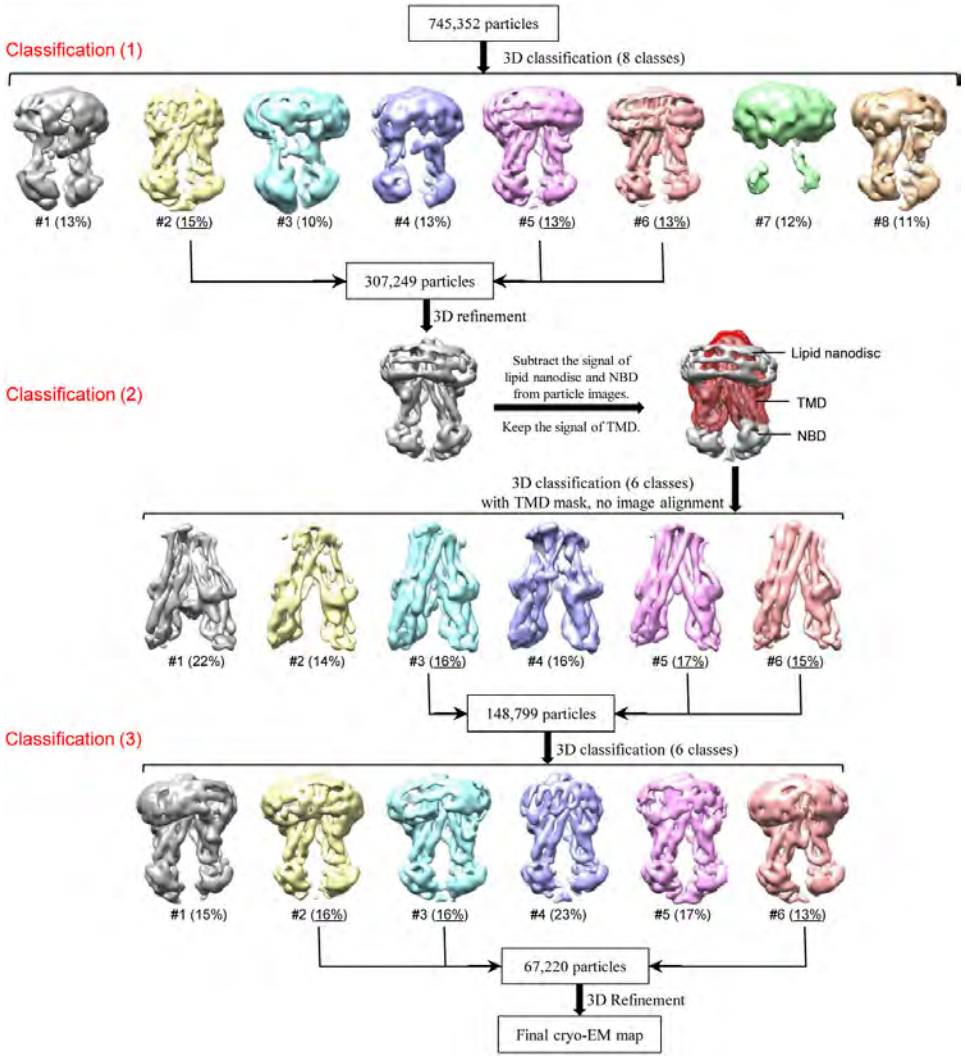
Nucleotide-free MsbA in nanodiscs
Cryo-EM and image processing

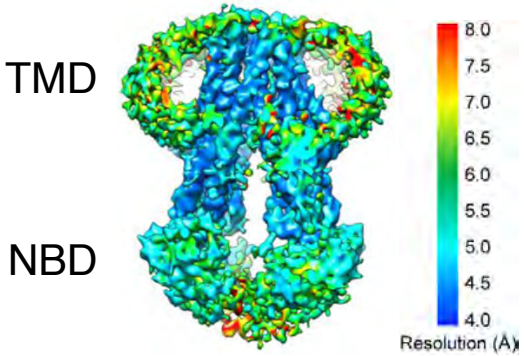




Mi *et al.* (2017) *Nature* 549: 233-237

Nucleotide-free MsbA in nanodiscs
Cryo-EM and image processing

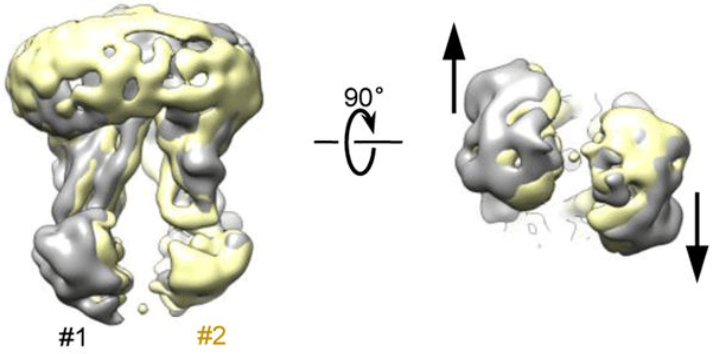




Resolution:

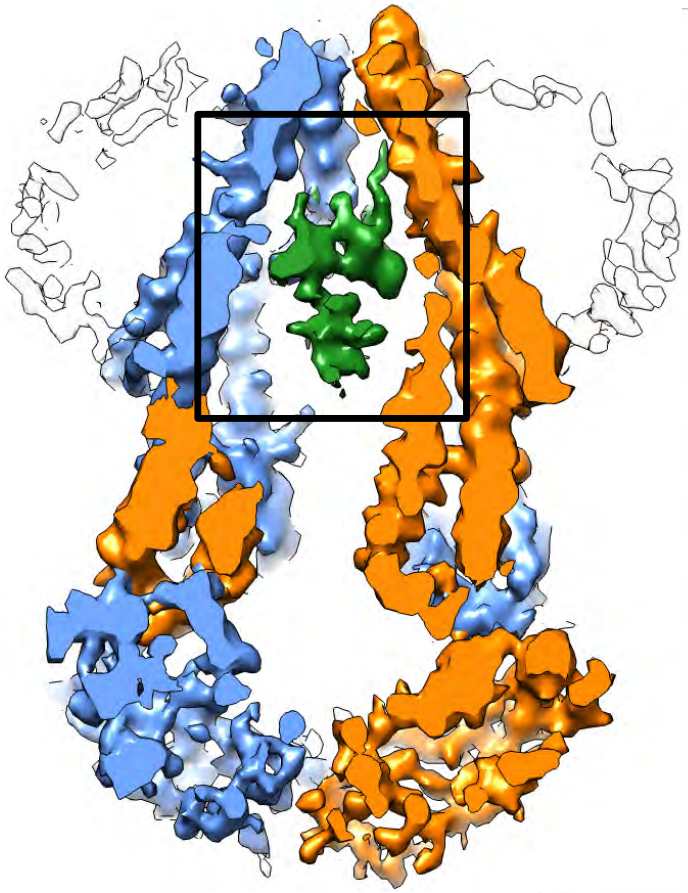
TMD+NBD = 4.7 Å

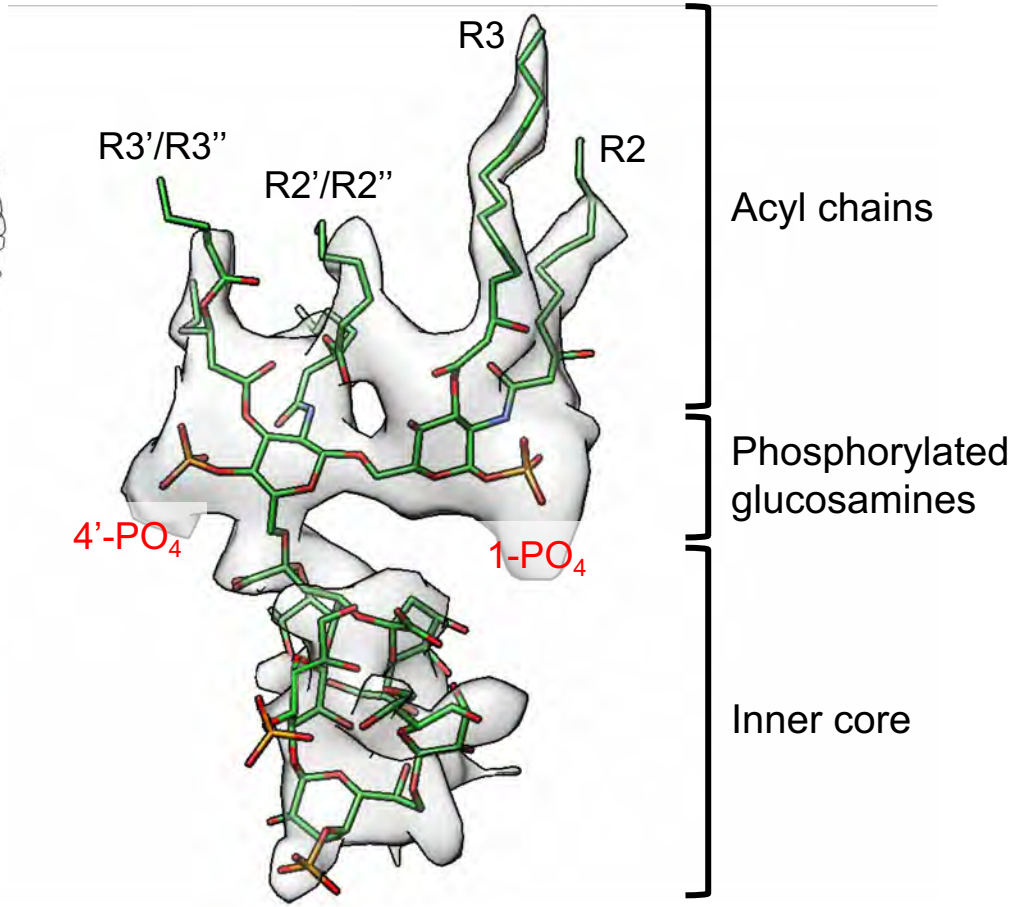
NBD only = 4.2 Å



Mi *et al.* (2017) *Nature* 549: 233-237

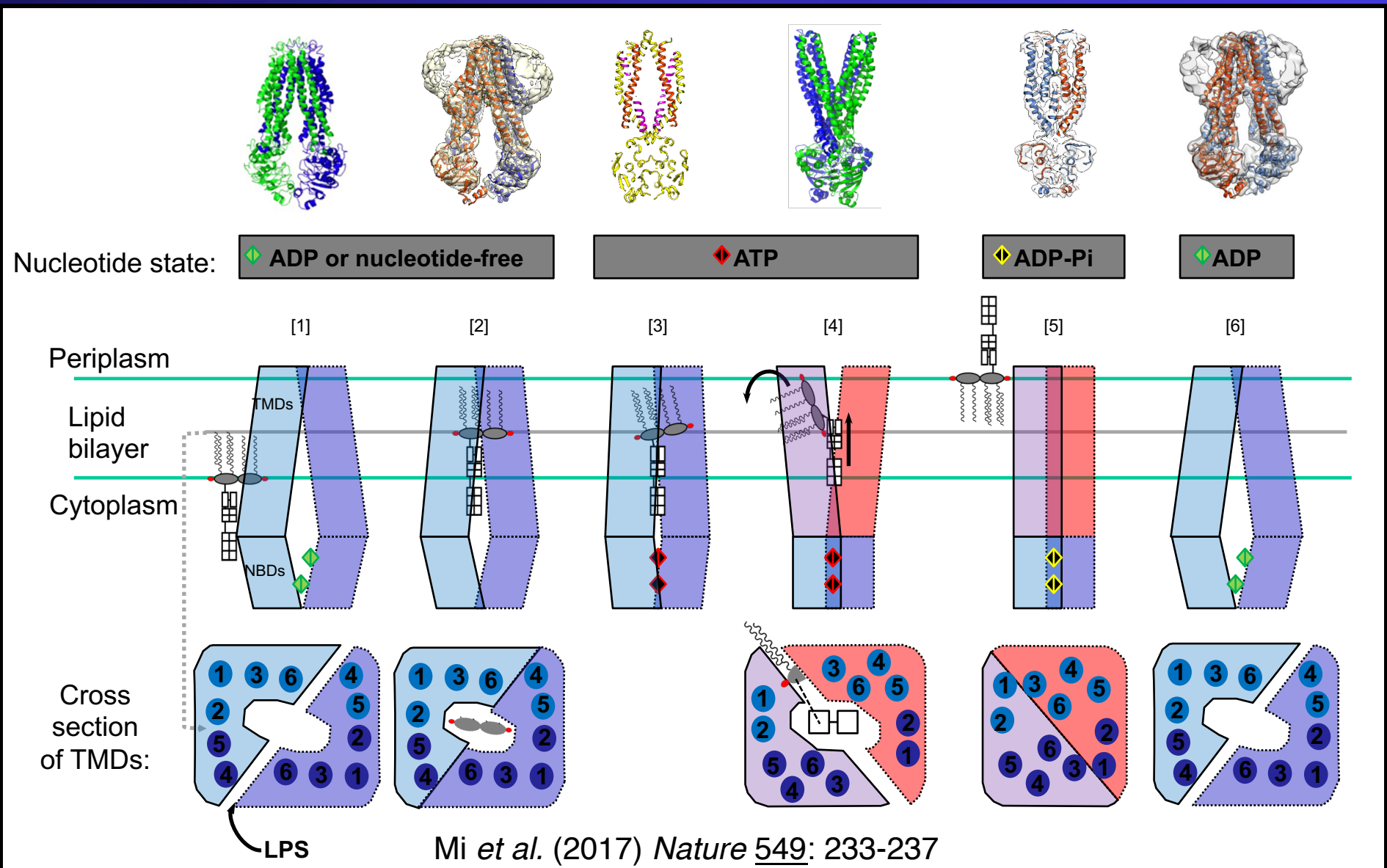
Map of nucleotide-free MsbA in nanodiscs shows density for LPS in the TMD





Mi *et al.* (2017) *Nature* 549: 233-237

Structures of MsbA in different functional states and mechanism of LPS flipping across membrane



Mi *et al.* (2017) *Nature* 549: 233-237

The peptidisc – a new membrane mimetic

eLIFE elifesciences.org

TOOLS AND RESOURCES

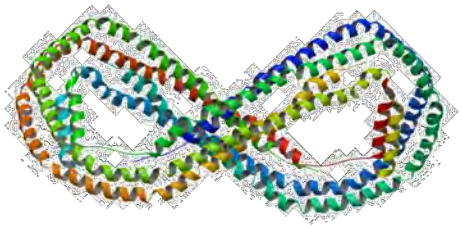
The Peptidisc, a simple method for stabilizing membrane proteins in detergent-free solution

Michael Luke Carlson[1], John William Young[1], Zhiyu Zhao[1], Lucien Fabre[1], Daniel Jun[2,3], Jianing Li[4], Jun Li[4], Harveer Singh Dhupar[1], Irvin Wason[1], Allan T Mills[1], J Thomas Beatty[3], John S Klassen[4], Isabelle Rouiller[2], Franck Duong[1]*

[1]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Life Sciences Institute, University of British Columbia, Vancouver, Canada; [2]Department of Anatomy and Cell Biology, McGill University, Montreal, Canada; [3]Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada; [4]Glycomics Centre and Department of Chemistry, University of Alberta, Alberta, Canada

Evolution of the peptidisc peptide

Apolipoprotein A1



Peptide 18A

DWLKAFYDKVAEKLKEAF

Model amphipathic peptide with similar lipid-associating properties as apolipoprotein A1

linked two 18A peptides with a proline residue

efficiently displaces apo-A1 from intact HDL

Peptide 18A-P-18A

DWLKAFYDKVAEKLKEAFPDWLKAFYDKVAEKLKEAF

Chung *et al.* (1985) *J. Biol. Chem.* 260: 10256-10262

substitute two leucines with phenylalanines

interacts better with lipids forms nanodiscs

NSP (Nanodisc Scaffold Peptide)

DWLKAFYDKVAEKLKEAAPDWFKAFYDKVAEKFKEAF

Kariyazono *et al.* (2016) *J. Pept Sci.* 22: 116-122

reverse amino acid sequence

increased solubility of the peptide

NSPr (Reversed NSP)

= Peptidisc peptide

FAEKFKEAVKDYFAKFWDPAAEKLKEAVKDYFAKLWD

Carlson *et al.* (2018) *eLife* 7: e34085

'On column' reconstitution of ABC transporter $MalFGK_2$ into peptidiscs

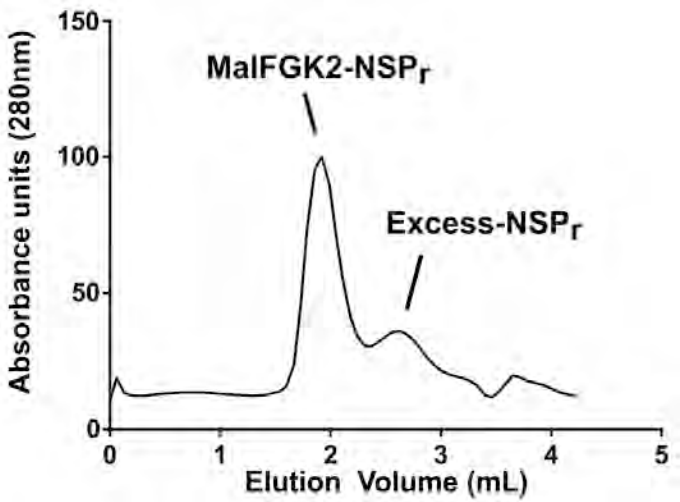
Approaches to reconstitute membrane proteins into peptidiscs:

– on column
– on beads
– on gradient

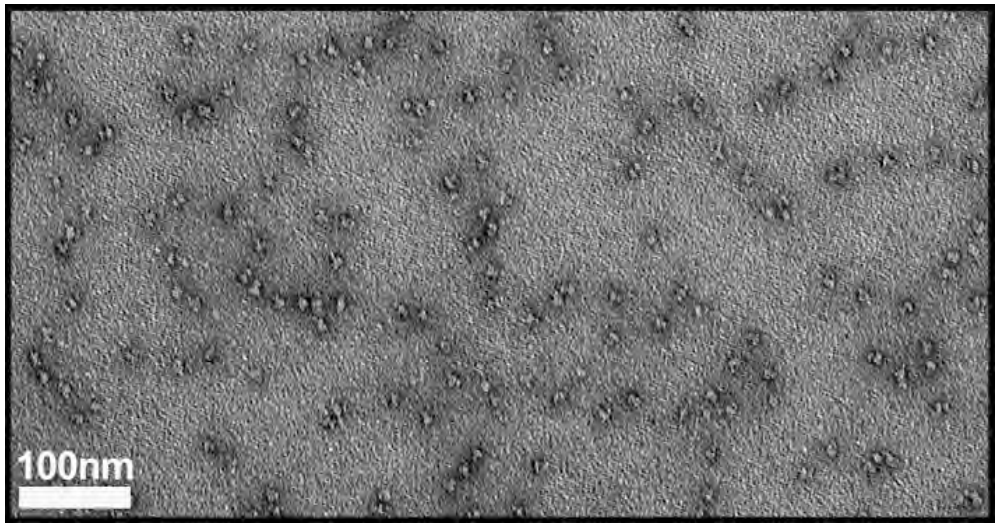
Carlson *et al.* (2018) *eLife* 7: e34085

'On column' reconstitution of ABC transporter $MalFGK_2$ into peptidiscs

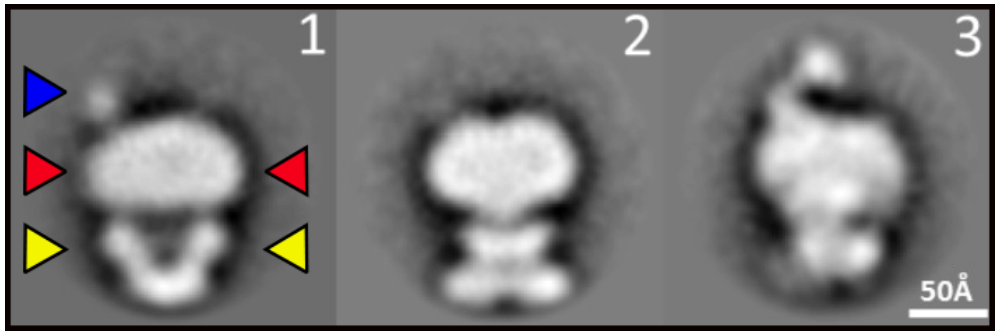
Size-exclusion chromatogram



Negative-stain EM image



2D class averages



Periplasmic P2 loop

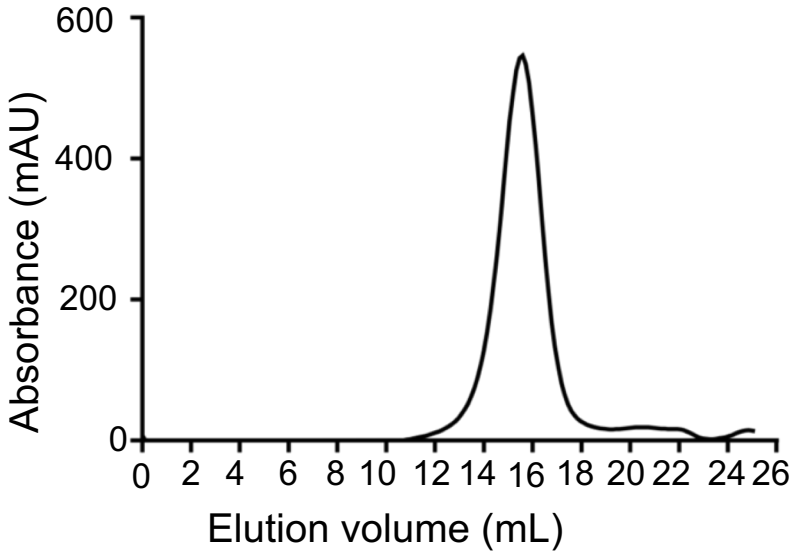
Transmembrane domain (MalFG)

Nucleotide-binding domain (MalK2)

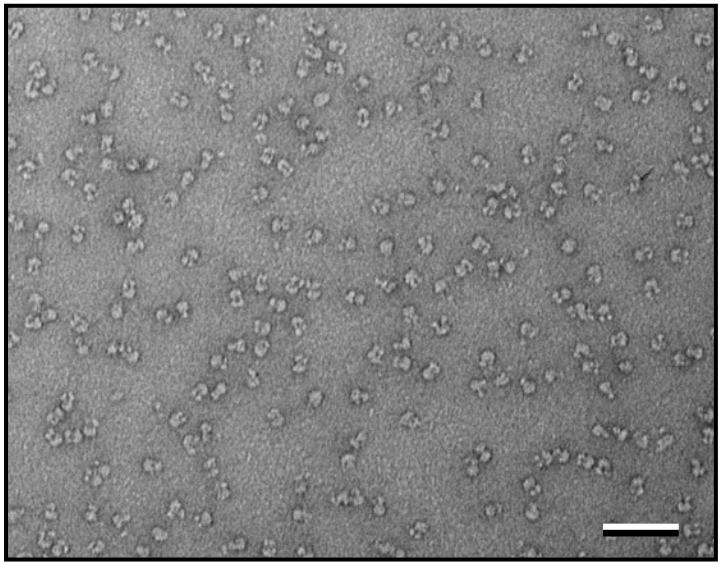
Carlson *et al.* (2018) *eLife* 7: e34085

'On column' reconstitution of nucleotide-free MsbA into peptidiscs

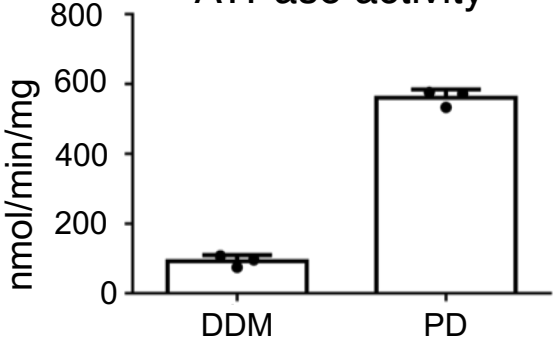
Size-exclusion chromatogram



Negative-stain EM image

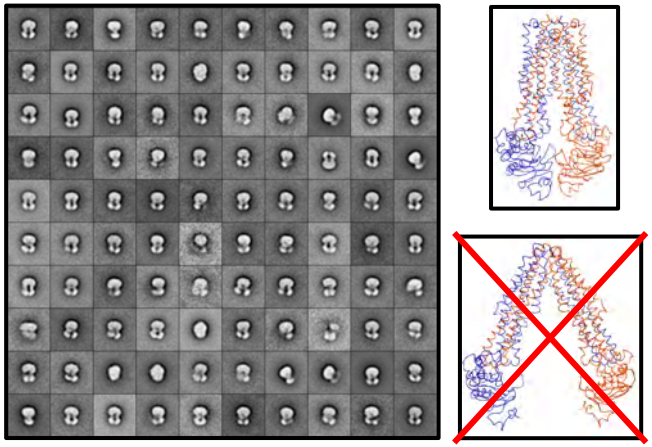


ATPase activity

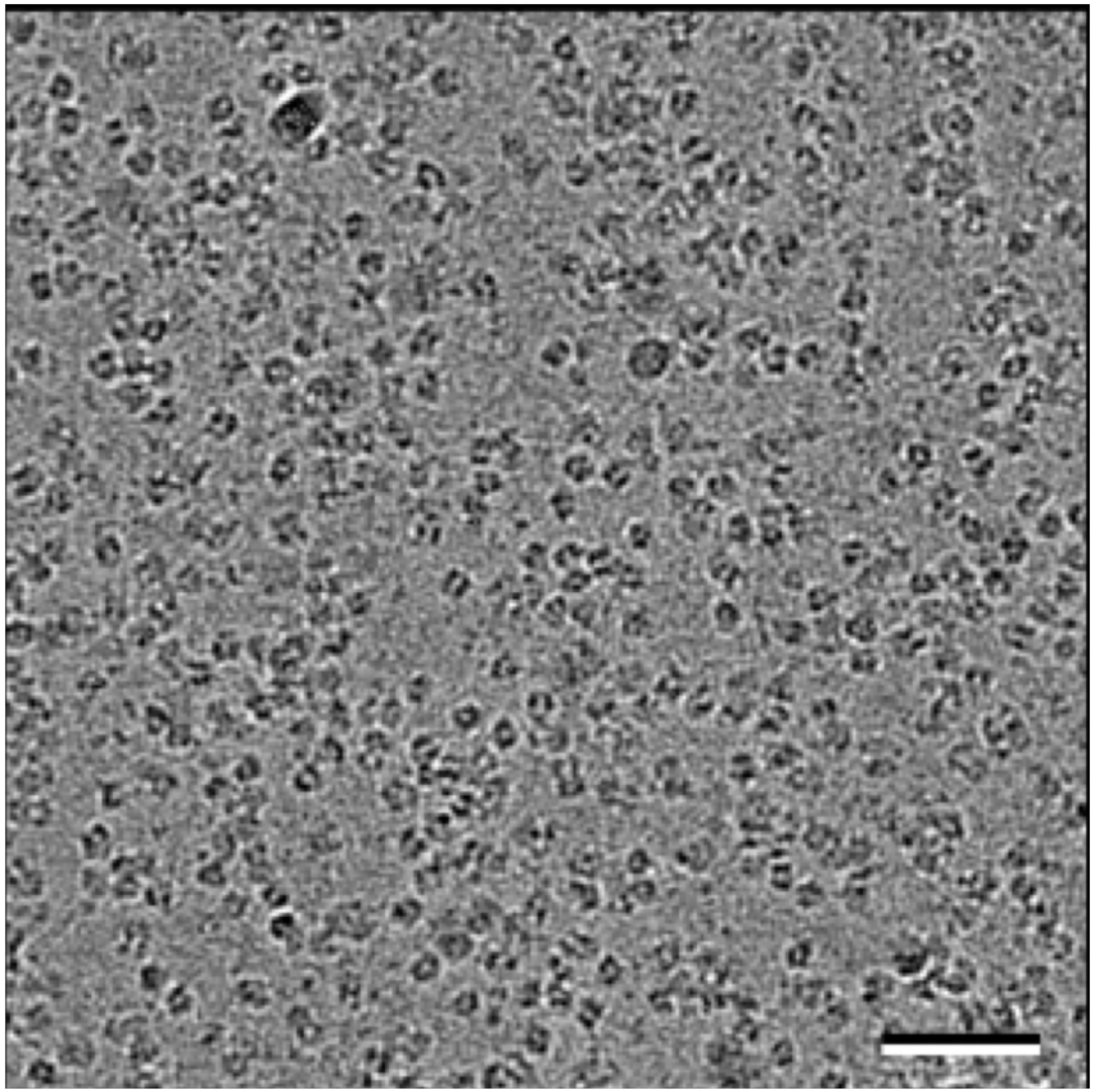


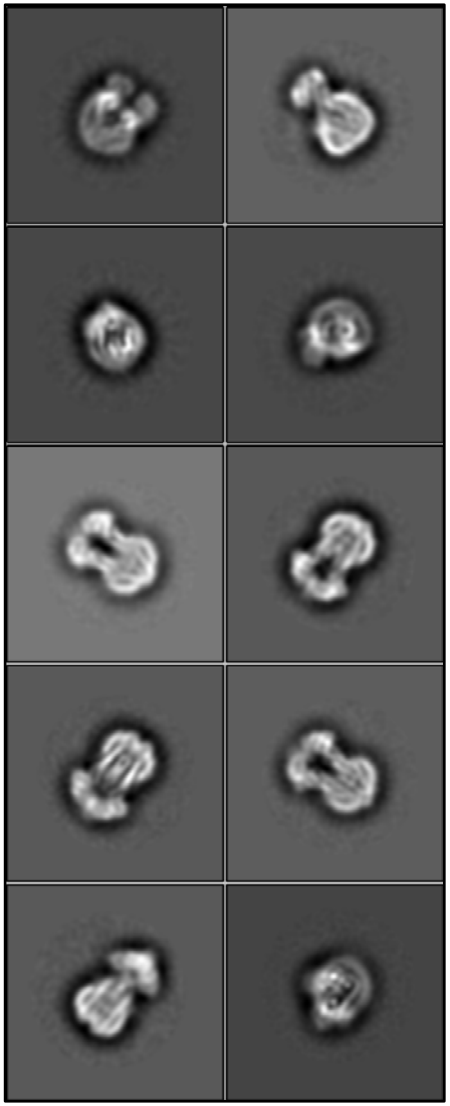
Peptidiscs stabilize MsbA as well as nanodiscs

2D class averages

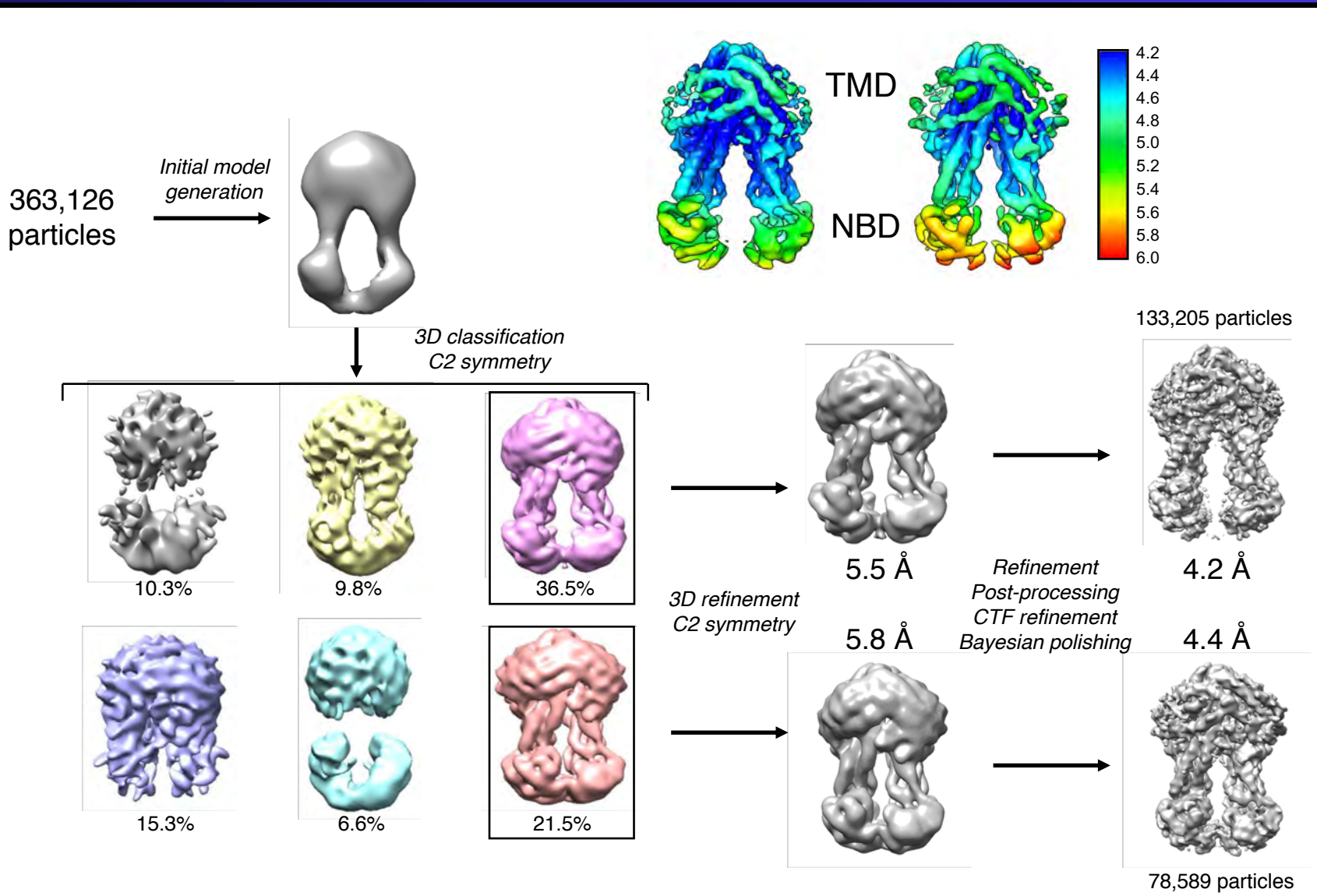


Nucleotide-free MsbA in peptidiscs
Cryo-EM and image processing

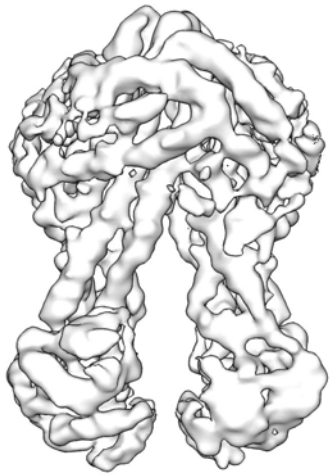




Nucleotide-free MsbA in peptidiscs
Cryo-EM and image processing

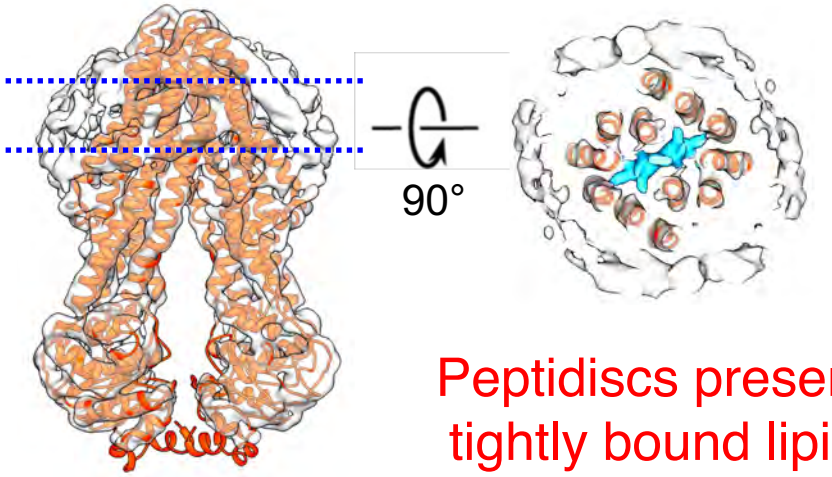


Nucleotide-free MsbA in peptidiscs
Cryo-EM and image processing

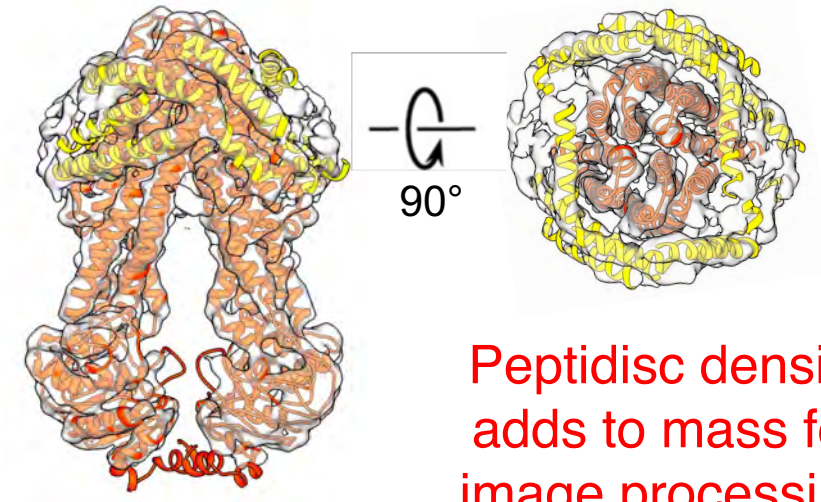


MsbA in peptidisc

MsbA in nanodisc

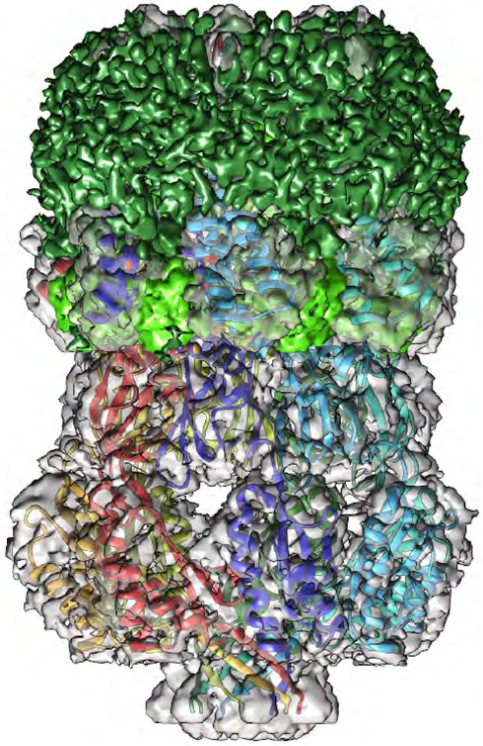


Peptidiscs preserve tightly bound lipids



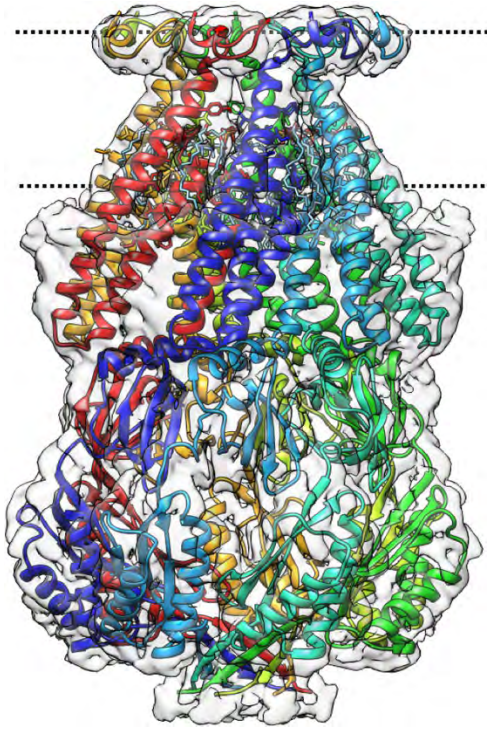
Peptidisc density adds to mass for image processing

Structures of *E. coli* MscS in nanodiscs



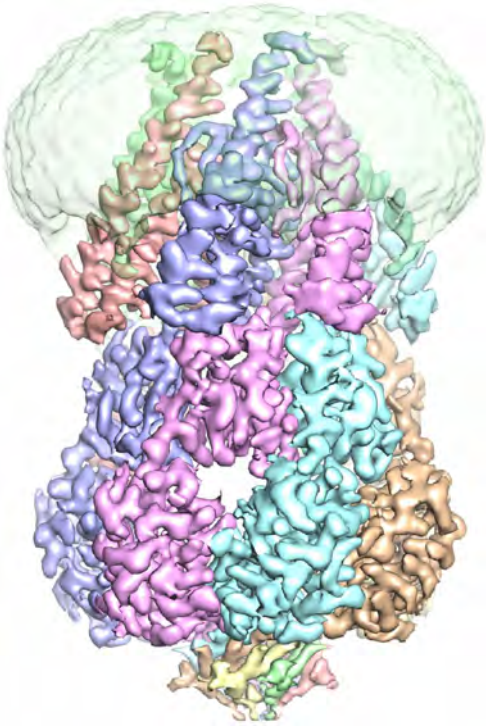
2.9 Å

Rasmussen *et al.* (2019)
J. Mol. Biol. 431: 3081-3090



3.1 Å

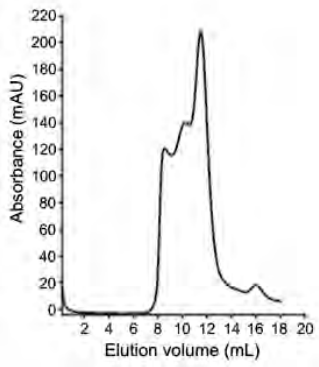
Reddy *et al.* (2019)
eLife 8: e50486



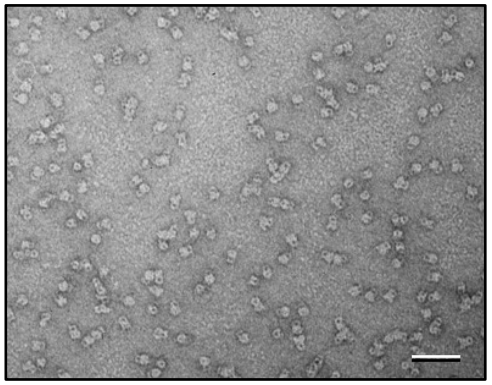
3.2 Å

Reconstitution of MscS into peptidiscs and structural analysis by cryo-EM

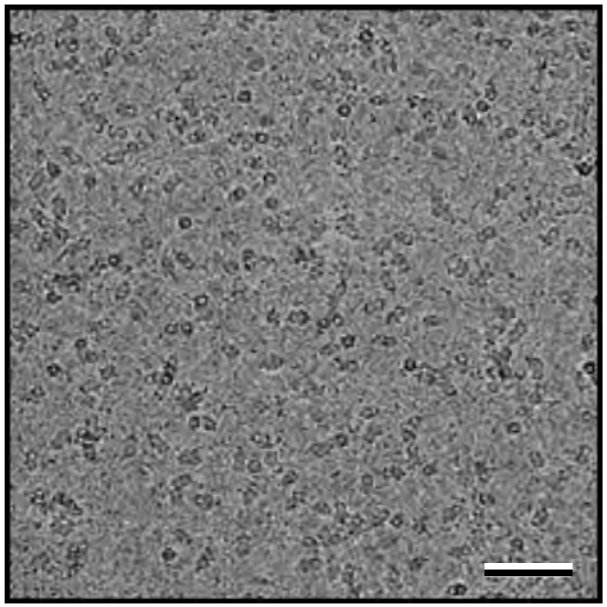
Size-exclusion chromatogram



Negative-stain EM image



Cryo-EM image



2D averages

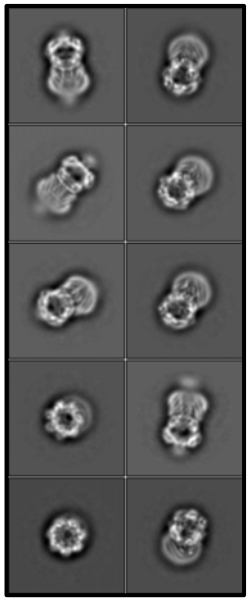
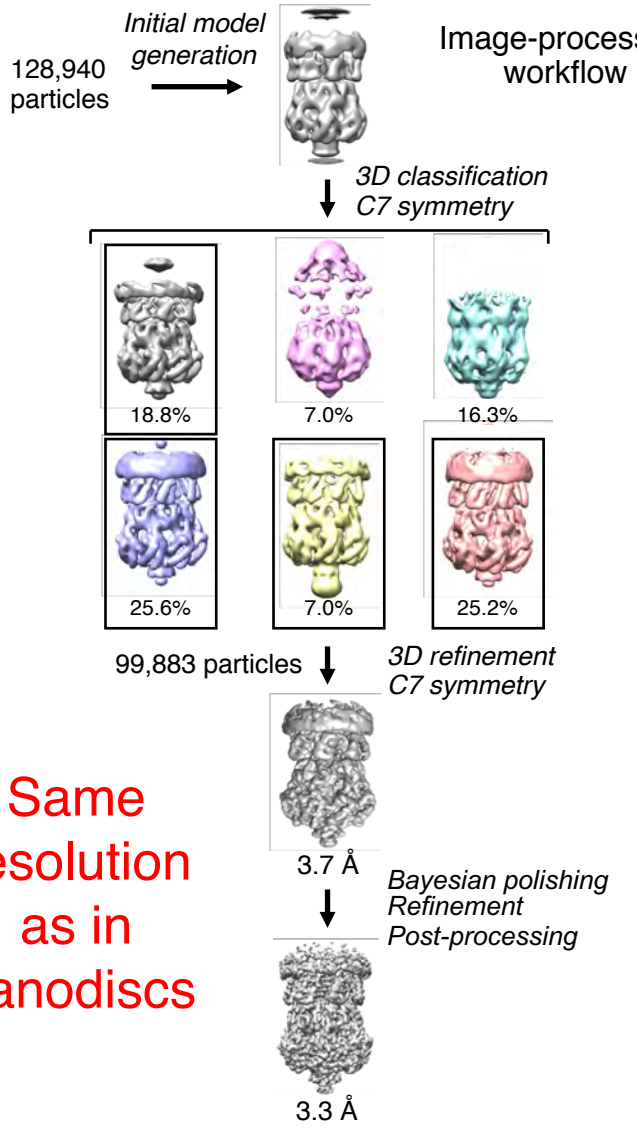
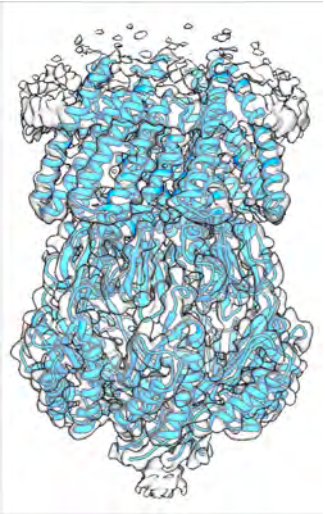


Image-processing workflow

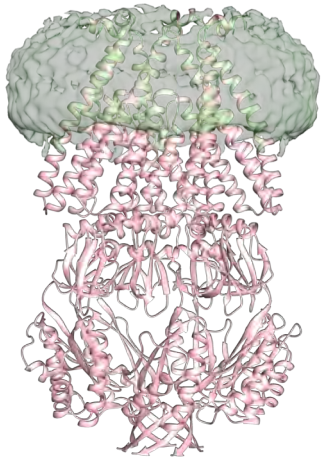


Same resolution as in nanodiscs

MscS in peptidiscs and nanodiscs

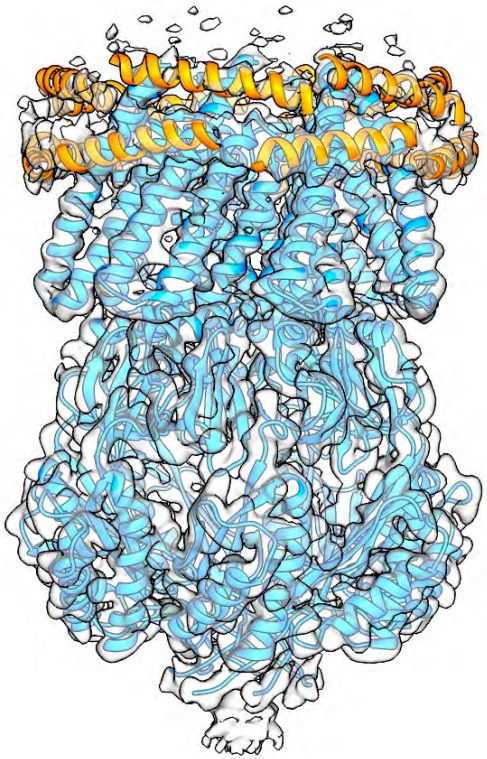


MscS in peptidisc

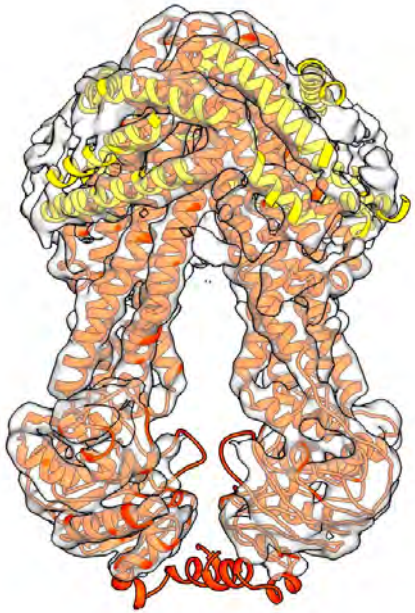


MscS in nanoidisc

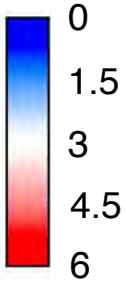
MscS in peptidisc

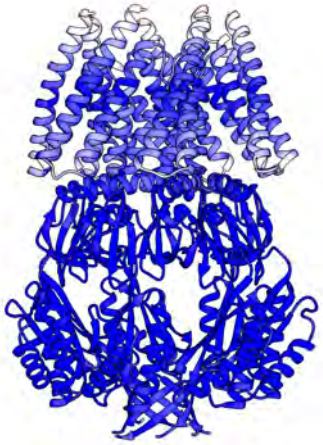


MsbA in peptidisc



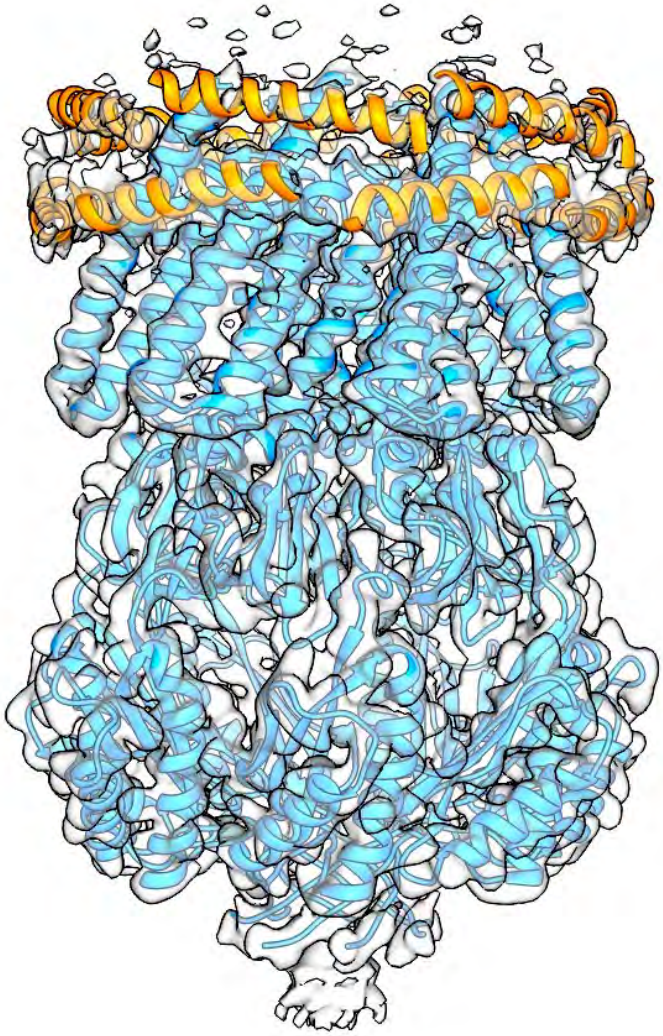
RMSD

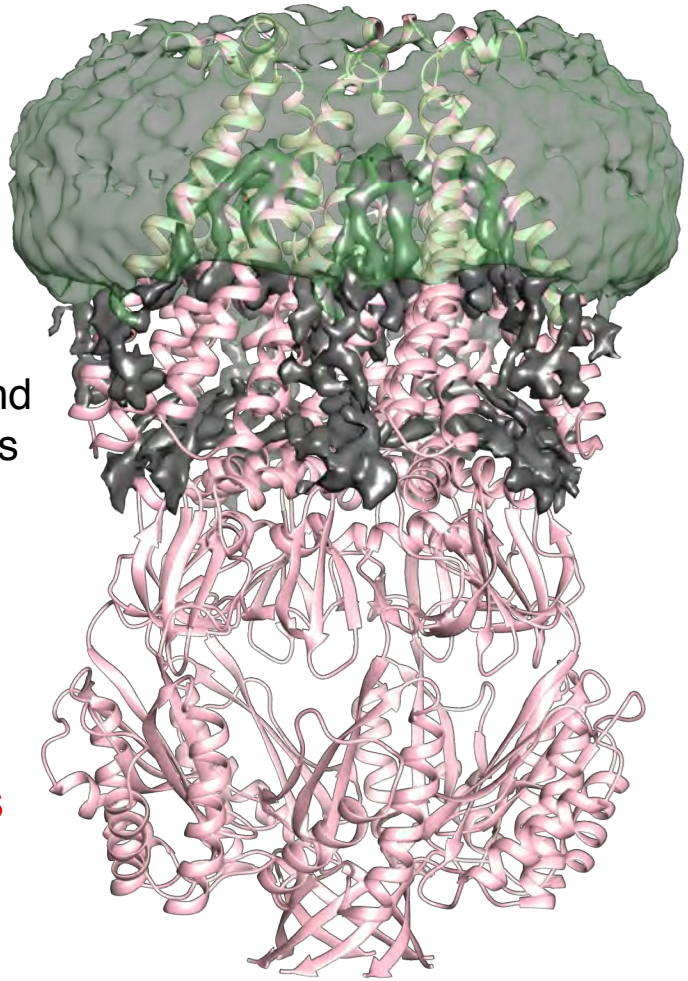




Peptidisc peptides adapt to
the TMDs of membrane proteins
in different ways

MscS in peptidiscs and nanodiscs

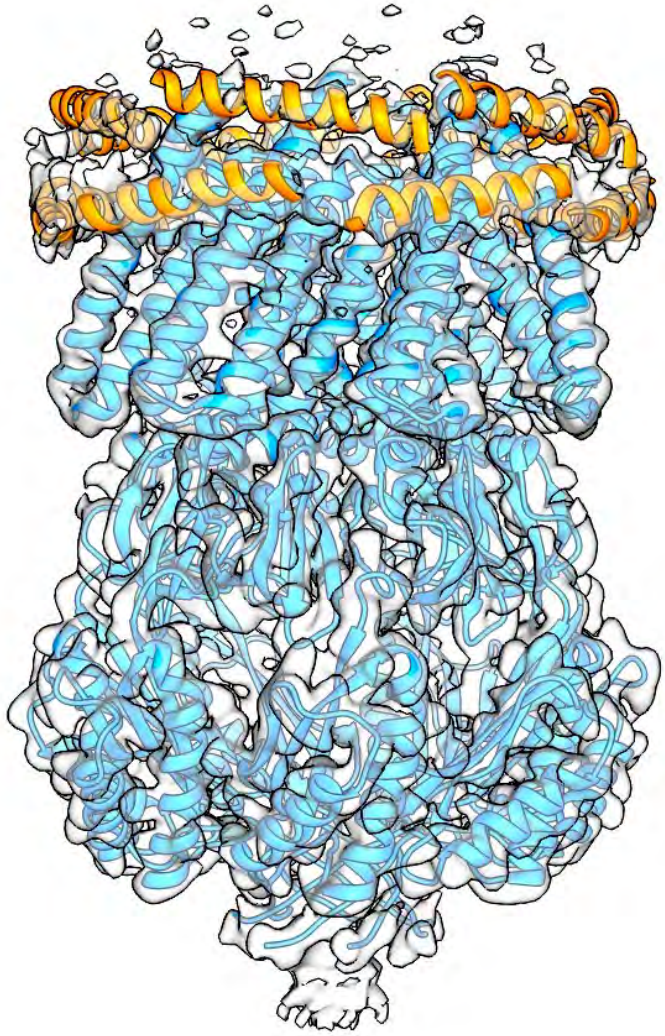


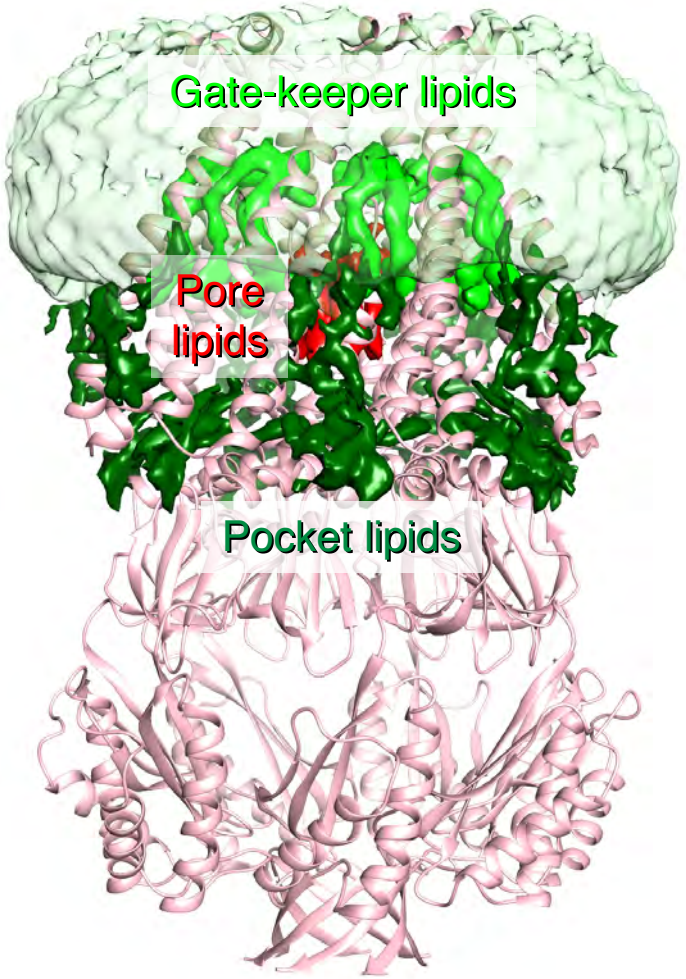


Bound lipids

Peptidiscs do not preserve loosely bound lipids

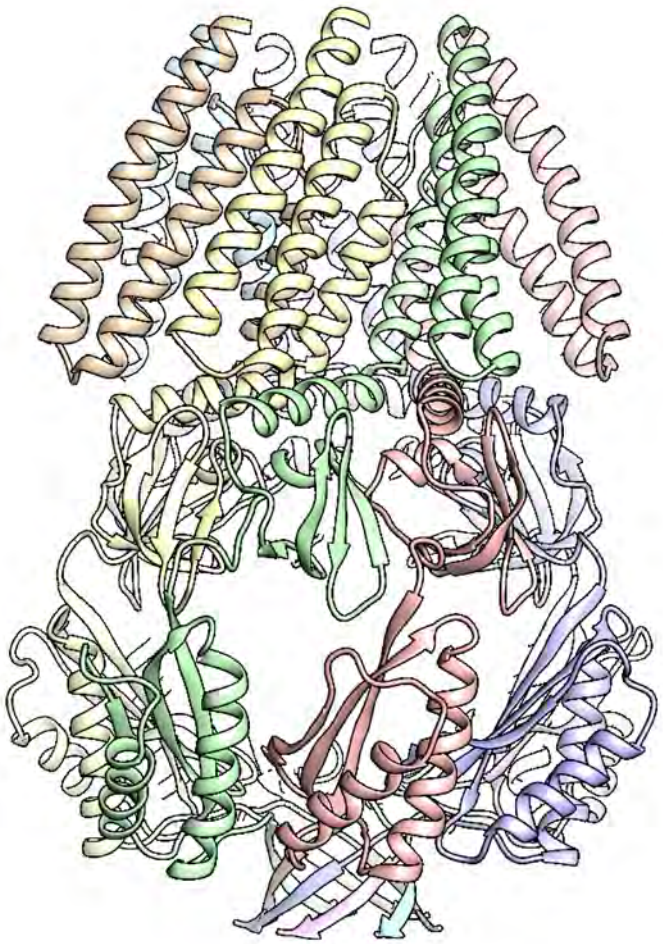
MscS in peptidiscs and nanodiscs



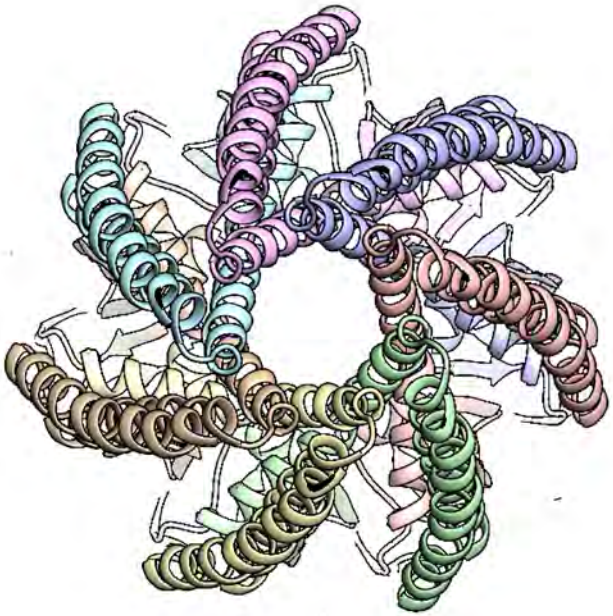


Conformational cycle of MscS

Closed



Closed



Membrane mimetics for cryo-EM studies

Detergents	– Most established – Risc of affecting MP structure – Can be used to modify orientation of particles
Peptidiscs	– Appears to stabilize MPs better than detergents – Excellent if only MP structure is of interest – Can induce formation of linear aggregates
Nanodiscs	– Excellent mimetic of biological membrane – Excellent if membrane characteristics are important – Problematic for MPs with little extramembranous mass
SMALPS	– Not much used yet for cryo-EM studies – Most native lipid environment – Structural heterogeneity ?

Acknowledgents

MsbA in nanodiscs

Harvard Medical School

Maofu Liao
Wei Mi

MscS in nanodiscs

Yixiao Zhang

MsbA and MscS in peptidiscs

Gabriella Angiulli
Hiroshi Suzuki

University of British Columbia

Franck Duong
Harveer Dhupar
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