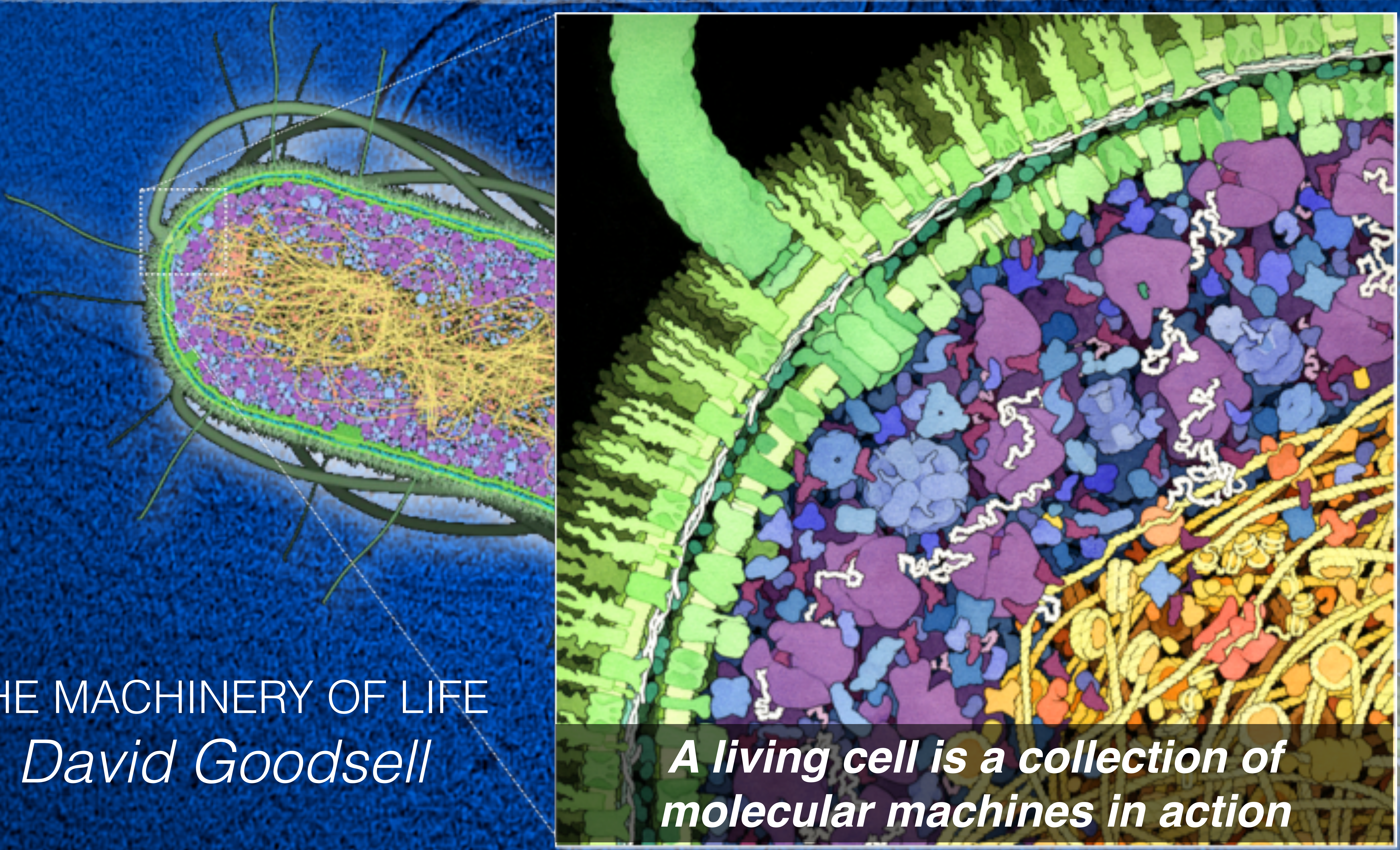
Workshop on Advanced Topics in EM Structure Determination: Challenges and Opportunities.
October 29 - November 3, 2017.
National Resource for Automated Molecular Microscopy
Simons Electron Microscopy Center, The New York Structural Biology Center

High-throughput cryo-electron tomography:
Visualizing Molecular Machines in Action

Jun Liu, PhD

Department of Microbial Pathogenesis
Microbial Sciences Institute
Yale School of Medicine

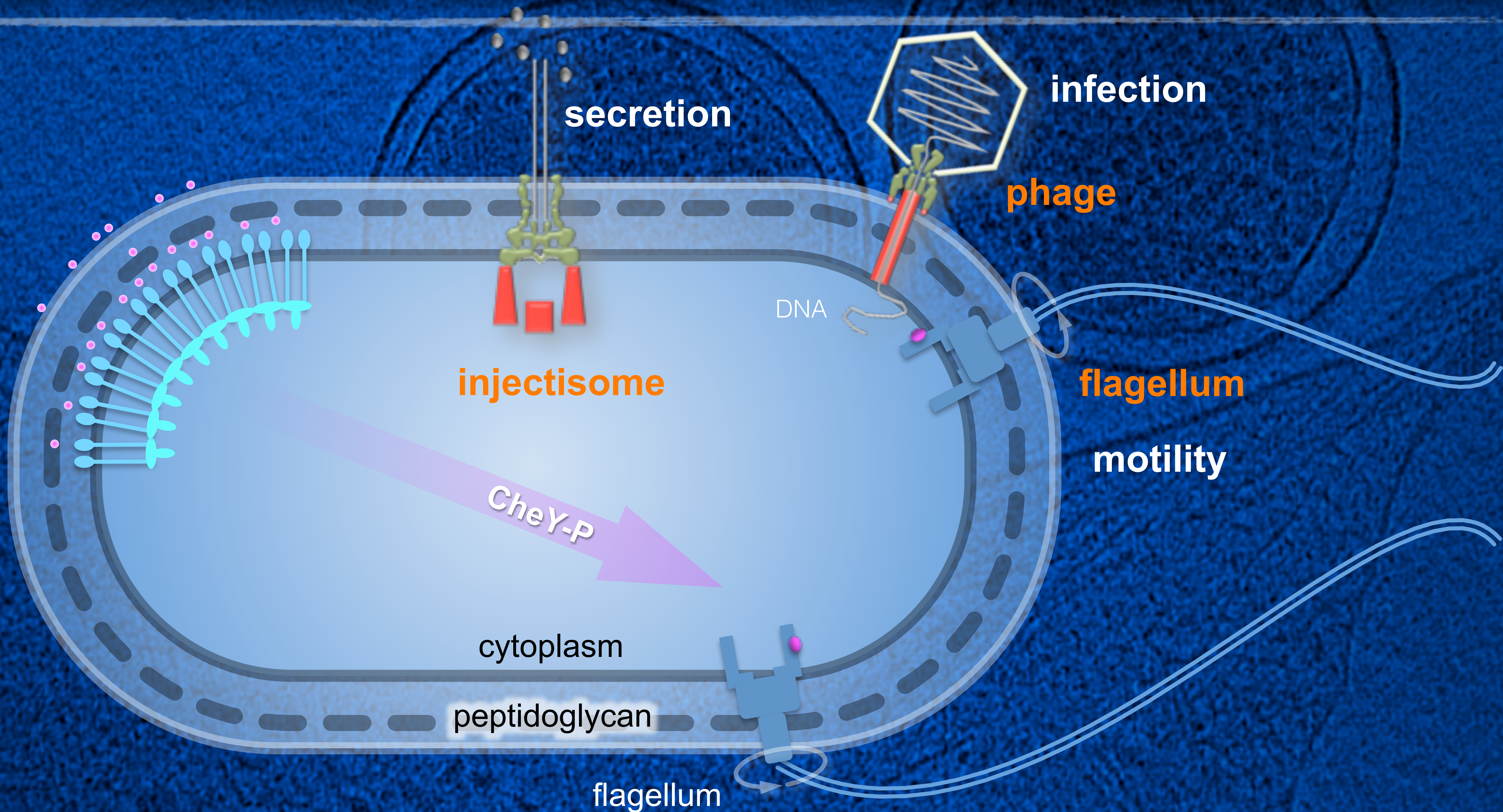
Motivated by the amazing illustration



THE MACHINERY OF LIFE

David Goodsell

Our interests: Bacterial nonomachines in action

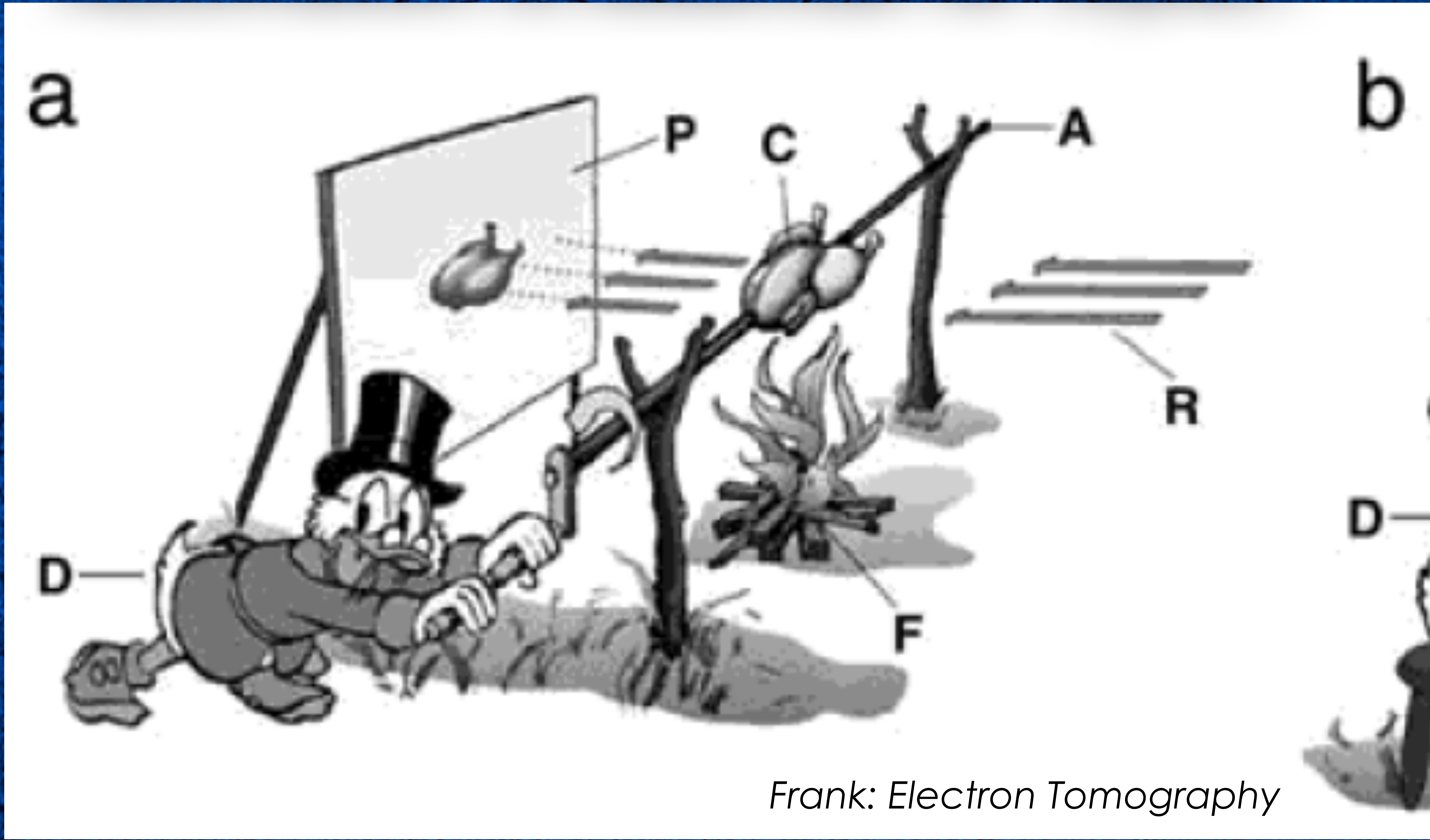


Rationale: These nanomachines play roles in bacterial pathogenesis

Outline

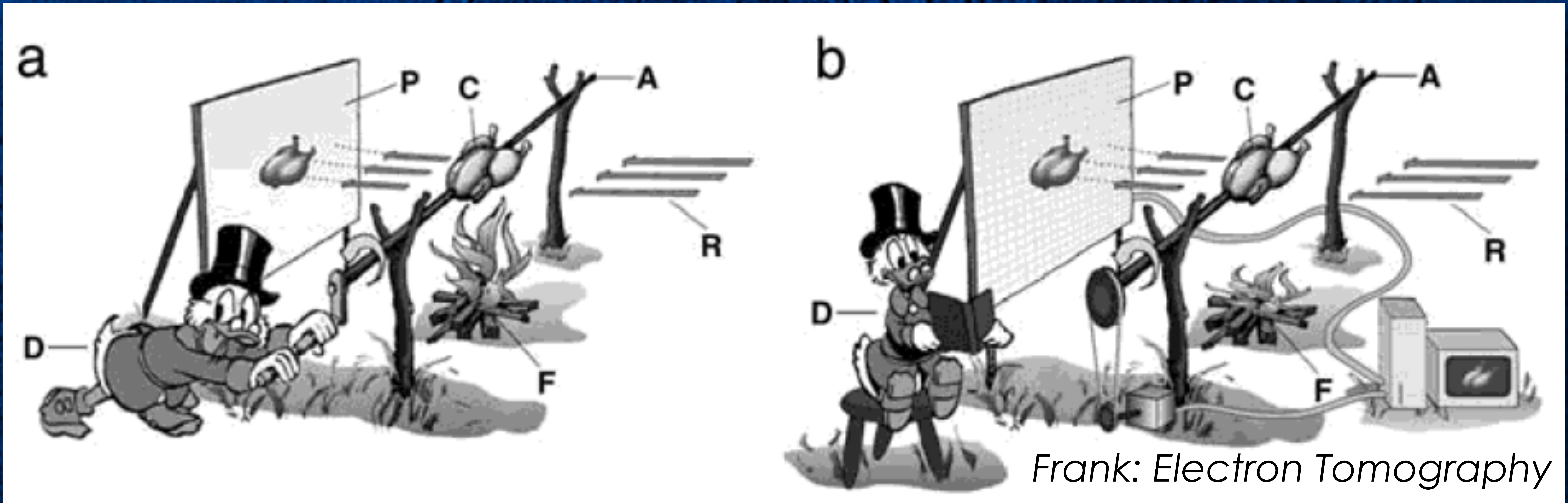
- ***Our techniques:***
  - *High-throughput cryo-ET pipeline*
  - *Production of bacterial minicells*
- ***Our systems:***
  - *Injectisomes in bacterial pathogens - Secretion*
  - *Phage infection - Trans-envelope channel formation*

Automation is essential for cryoET



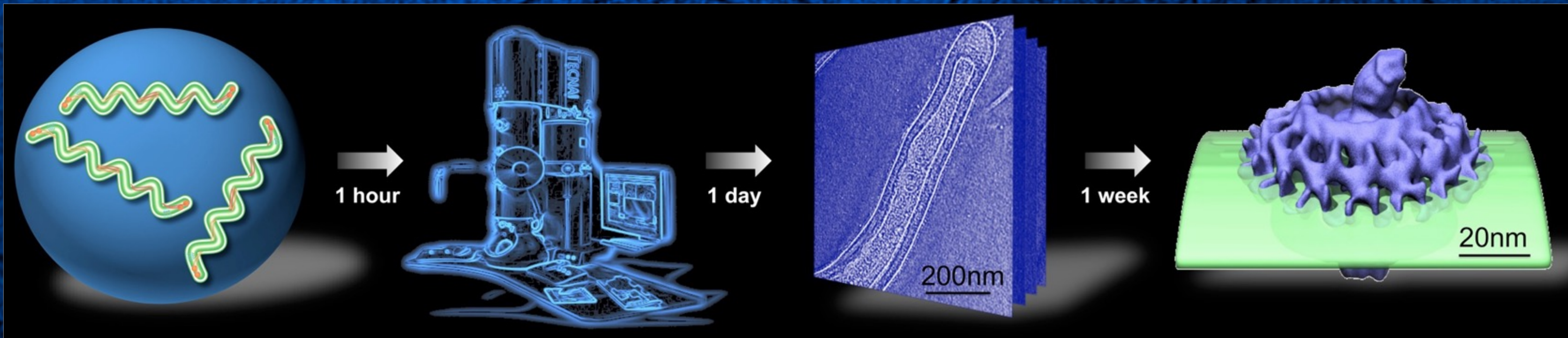
Frank: Electron Tomography

Automation is essential for cryoET



Frank: Electron Tomography

High throughput cryo-electron tomography



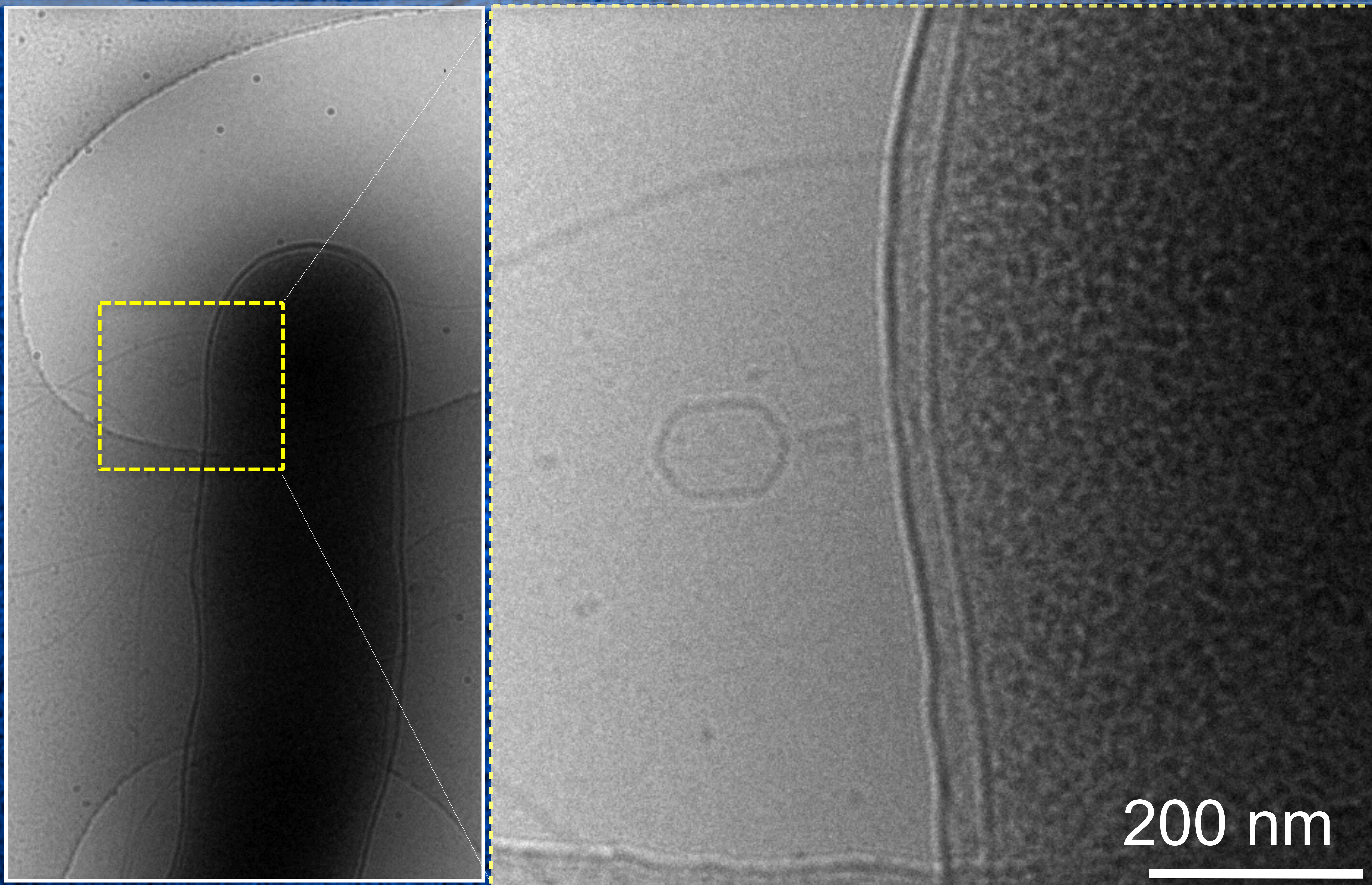
SerialEM —> MotionCor2 —> IMOD —> Tomo3D —> I3

Samples —TOMOAUTO→ **3-D Tomograms**

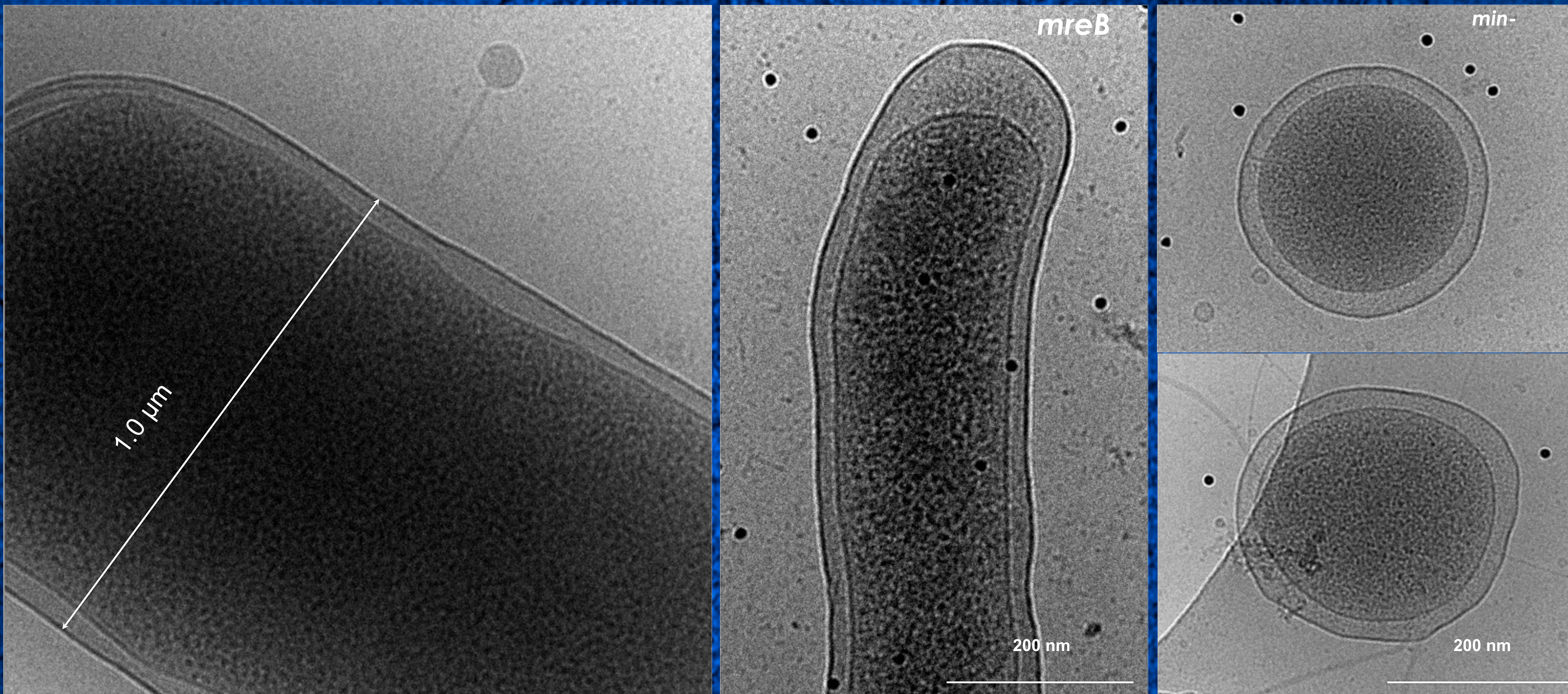
In three days: 360 tilt series (41x8 frames); 118,080 2-D images; 4.0 Tb raw data; 32.0 Tb 3-D Tomograms.

Morado et al. JoVE 2016   Hu et al. PNAS 2015

A typical bacterium is too large for cryo-ET



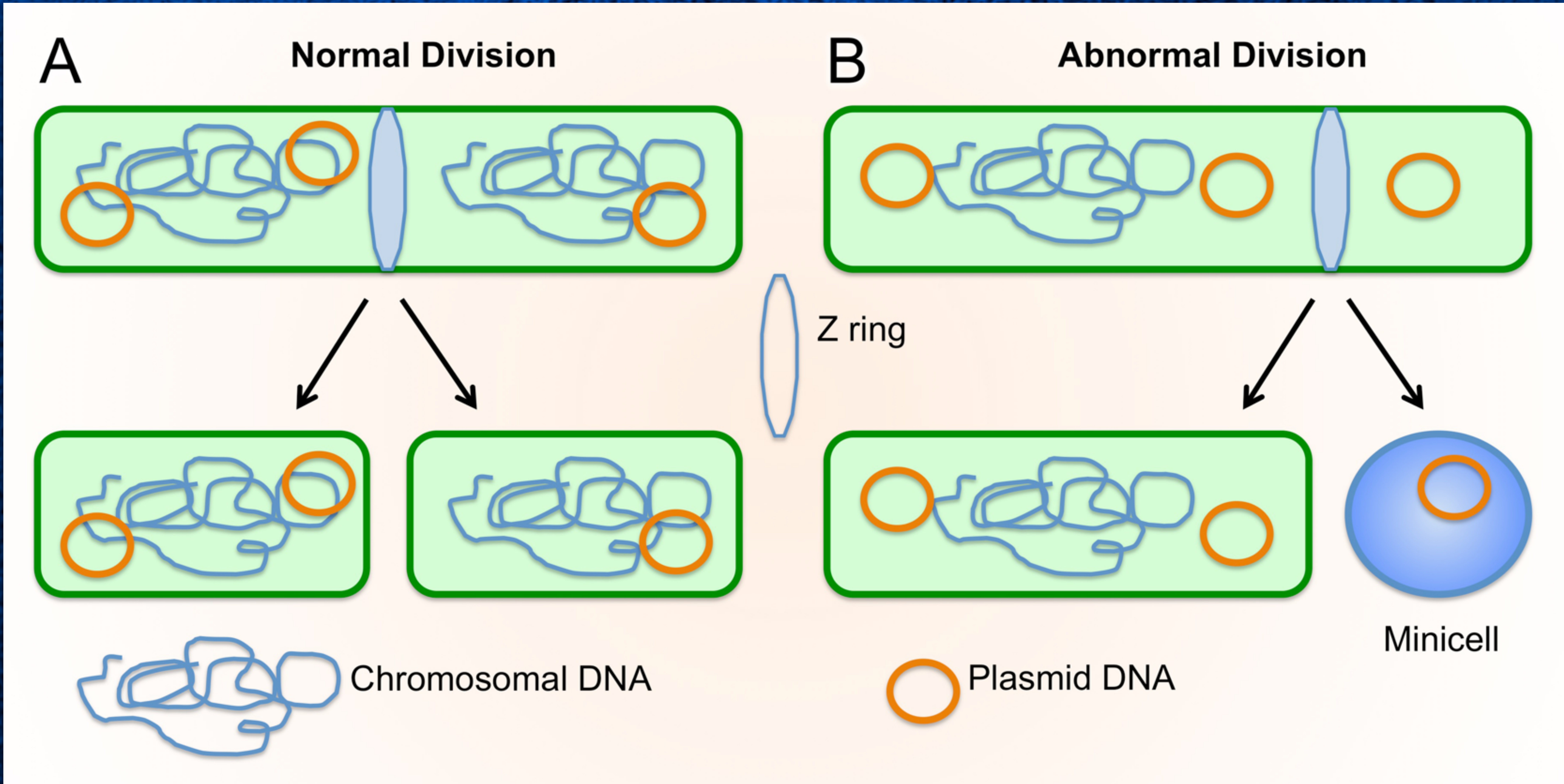
Our solution: bacterial minicells



In collaboration with
Dr. Bill Margolin

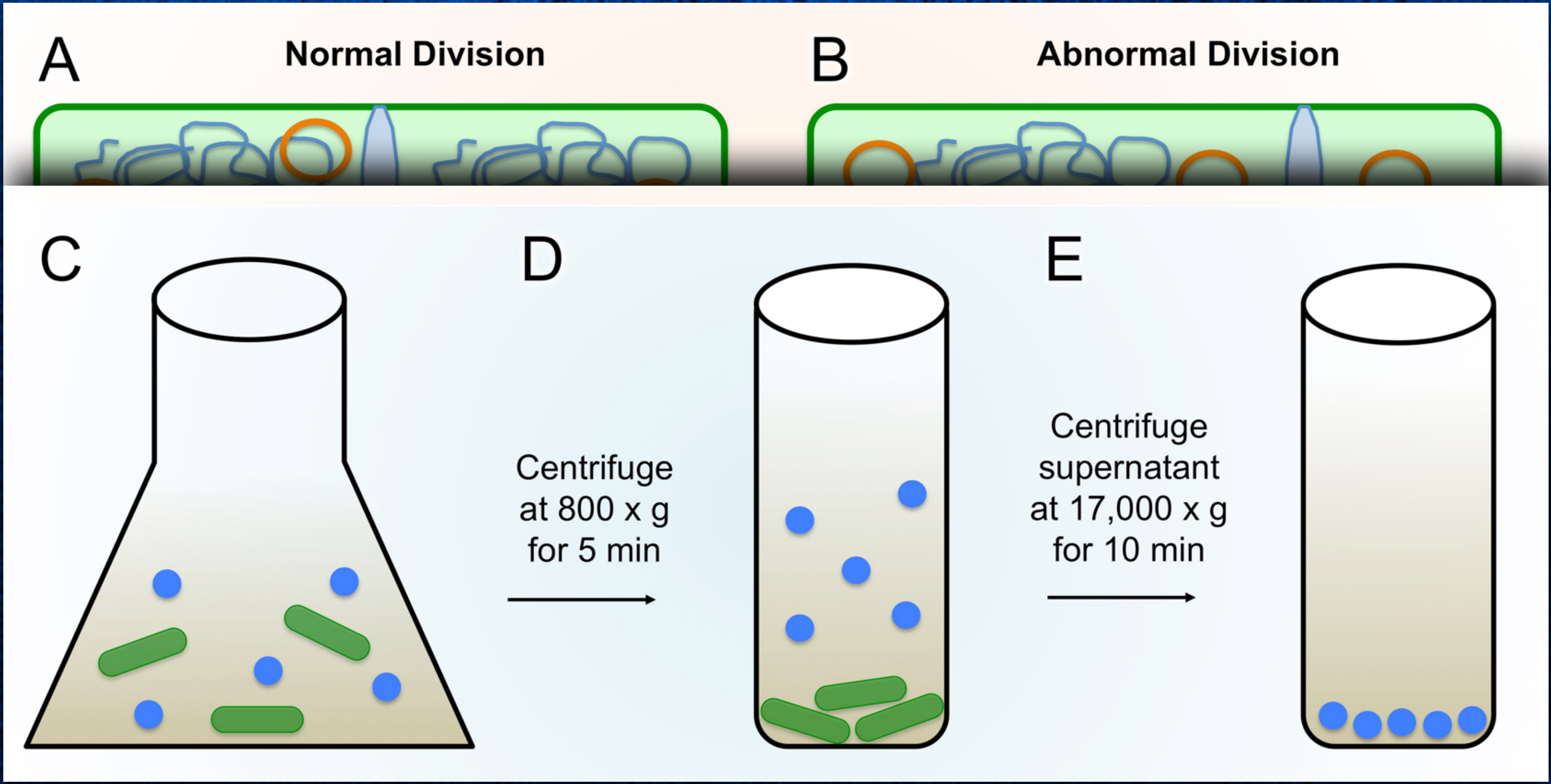
Liu et al. Virology (2011)
Liu et al. PNAS (2012)

Production of minicells



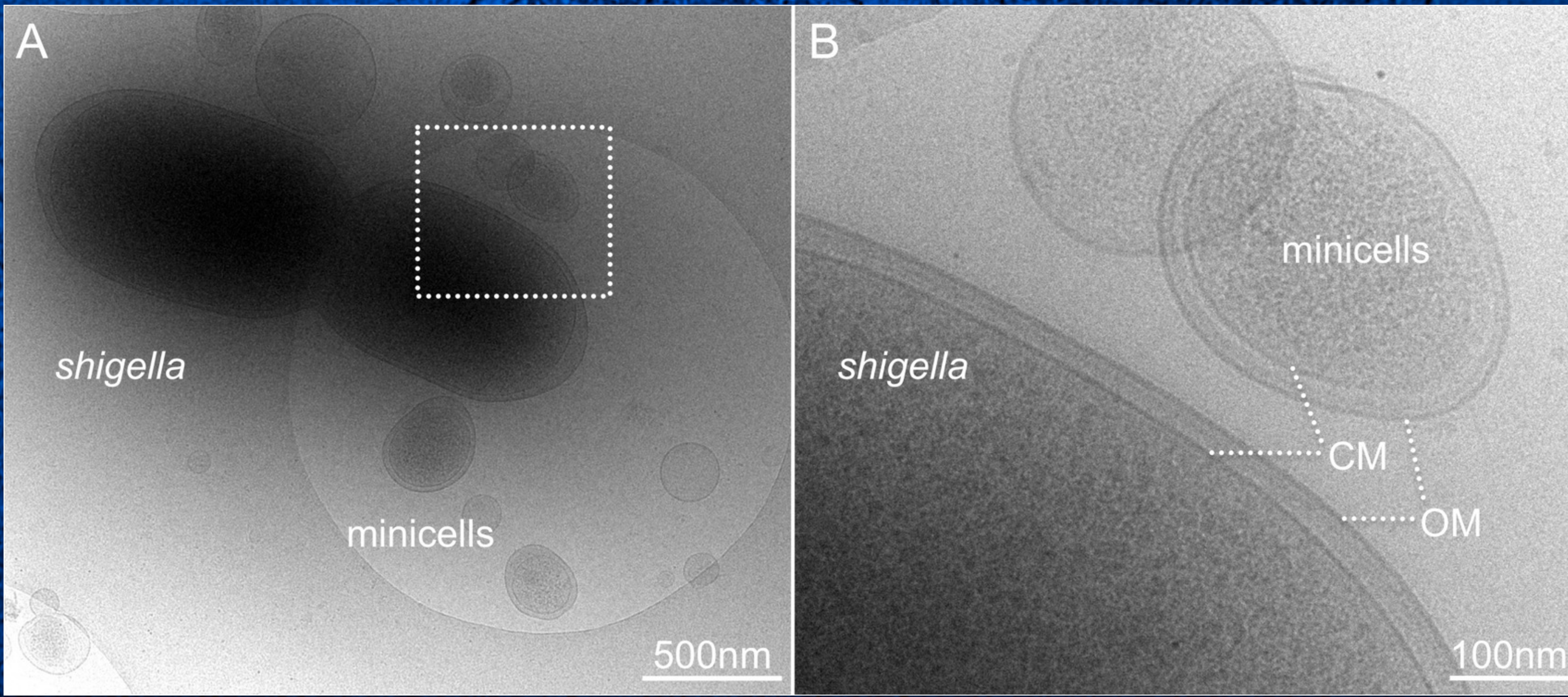
Farley et al., 2016; Carleton et al., 2013

Production of minicells



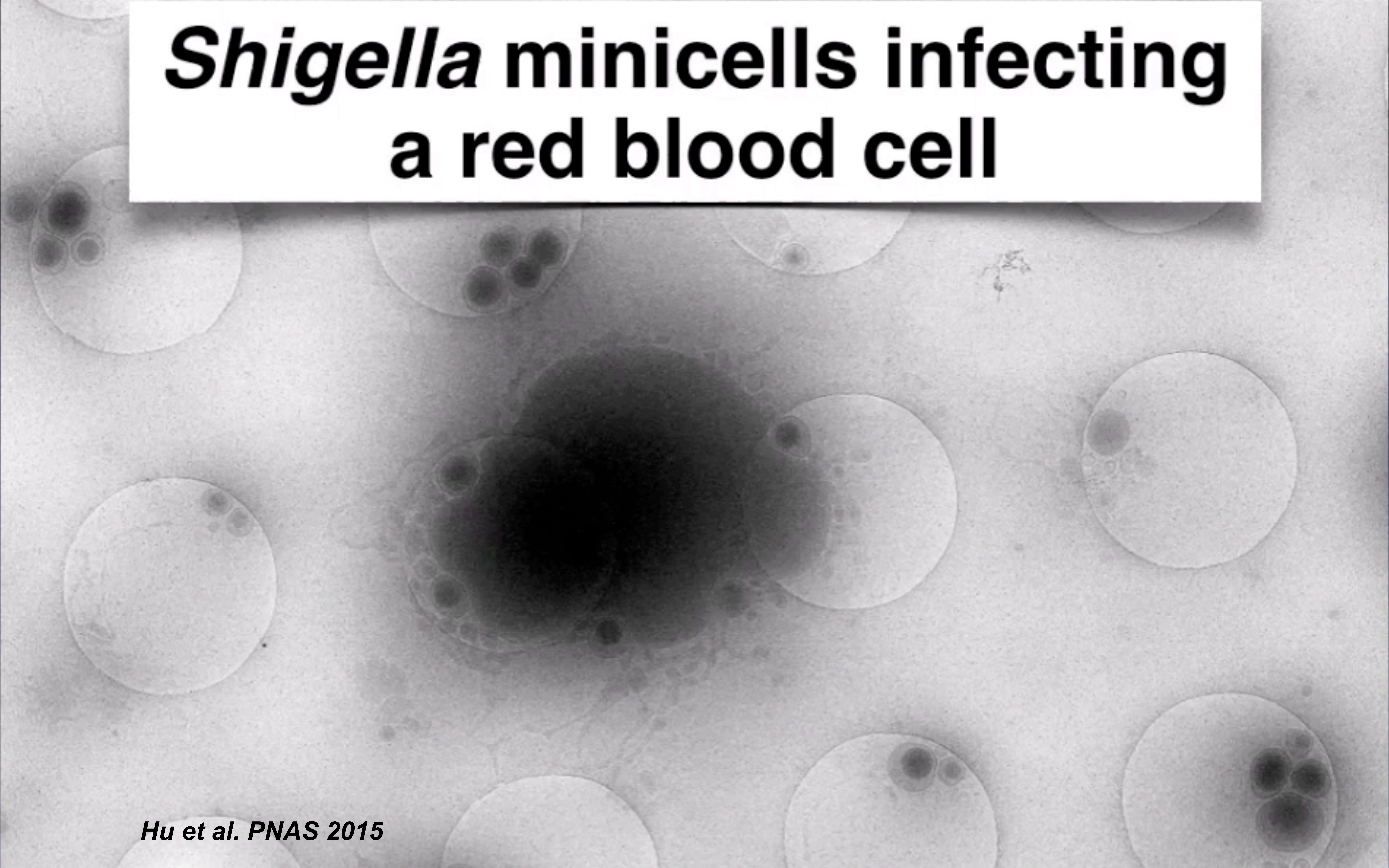
Farley et al., 2016; Carleton et al., 2013

Shigella minicells



Hu et al. PNAS 2015

Shigella minicells infecting a red blood cell



Hu et al. PNAS 2015

Bacterial nanomachines (I)

Bacterial type III secretion systems in *Shigella* & *Salmonella*

Collaborators:
Maria Lara-Tejero, Jorge Galan (Yale)
Bill Picking (Kansas)

Supported by NIH (R01 AI123351)

Host-Pathogen Interactions

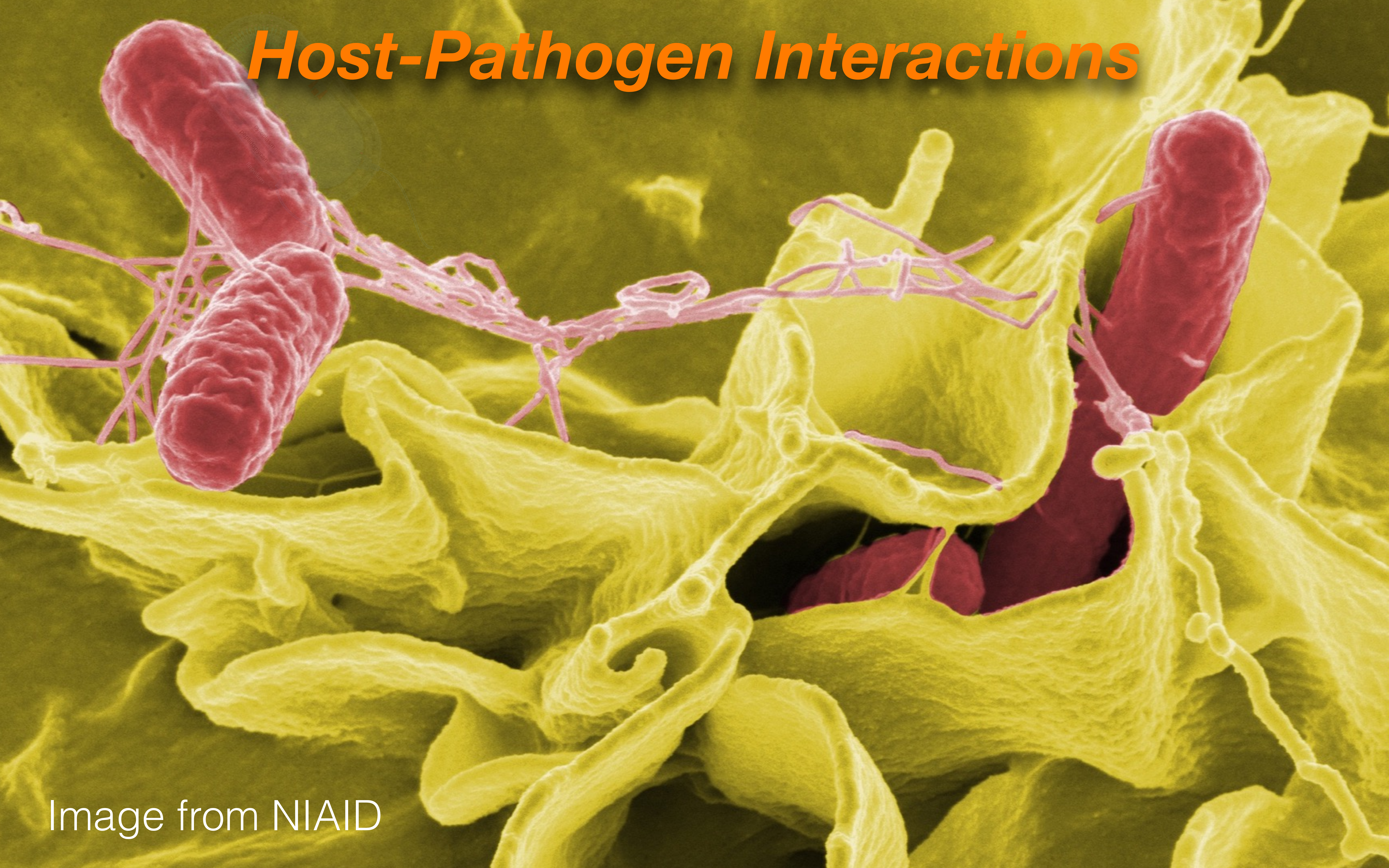
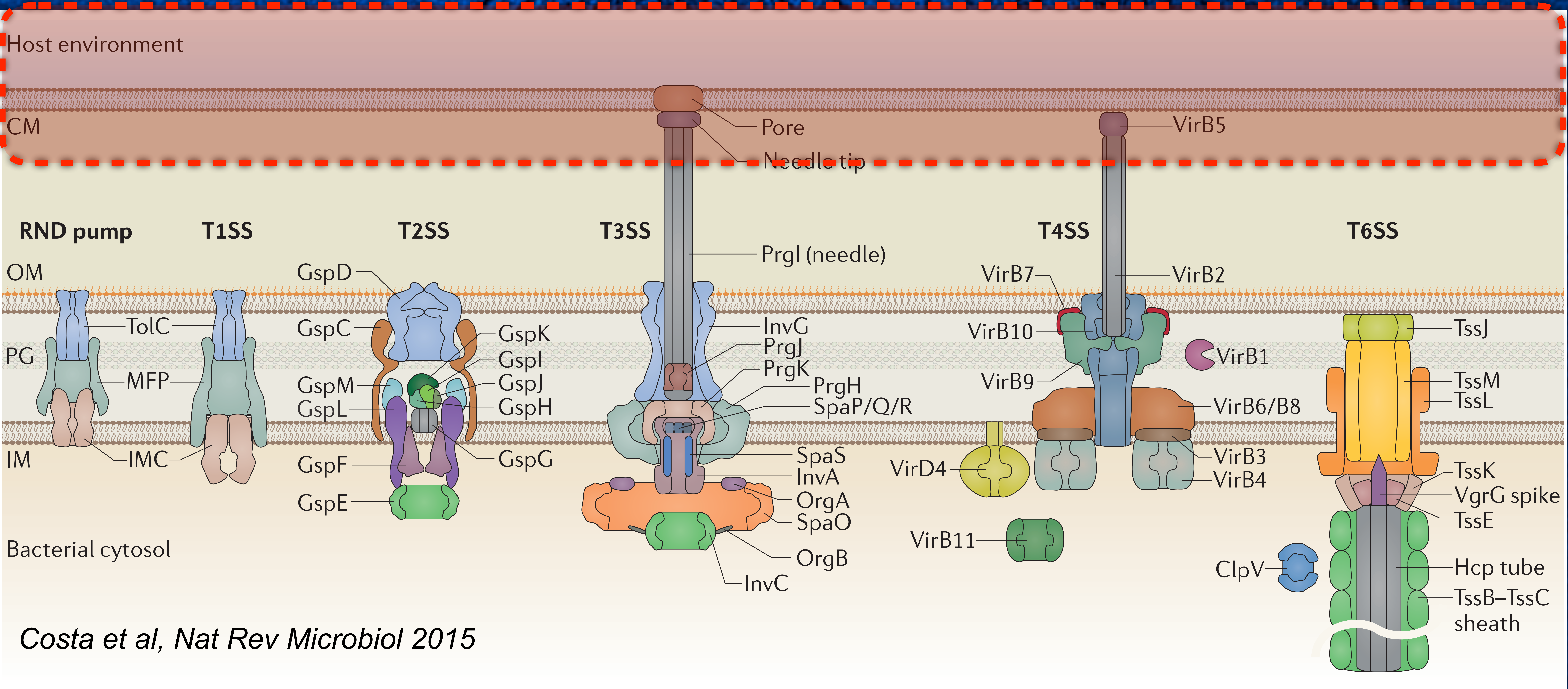


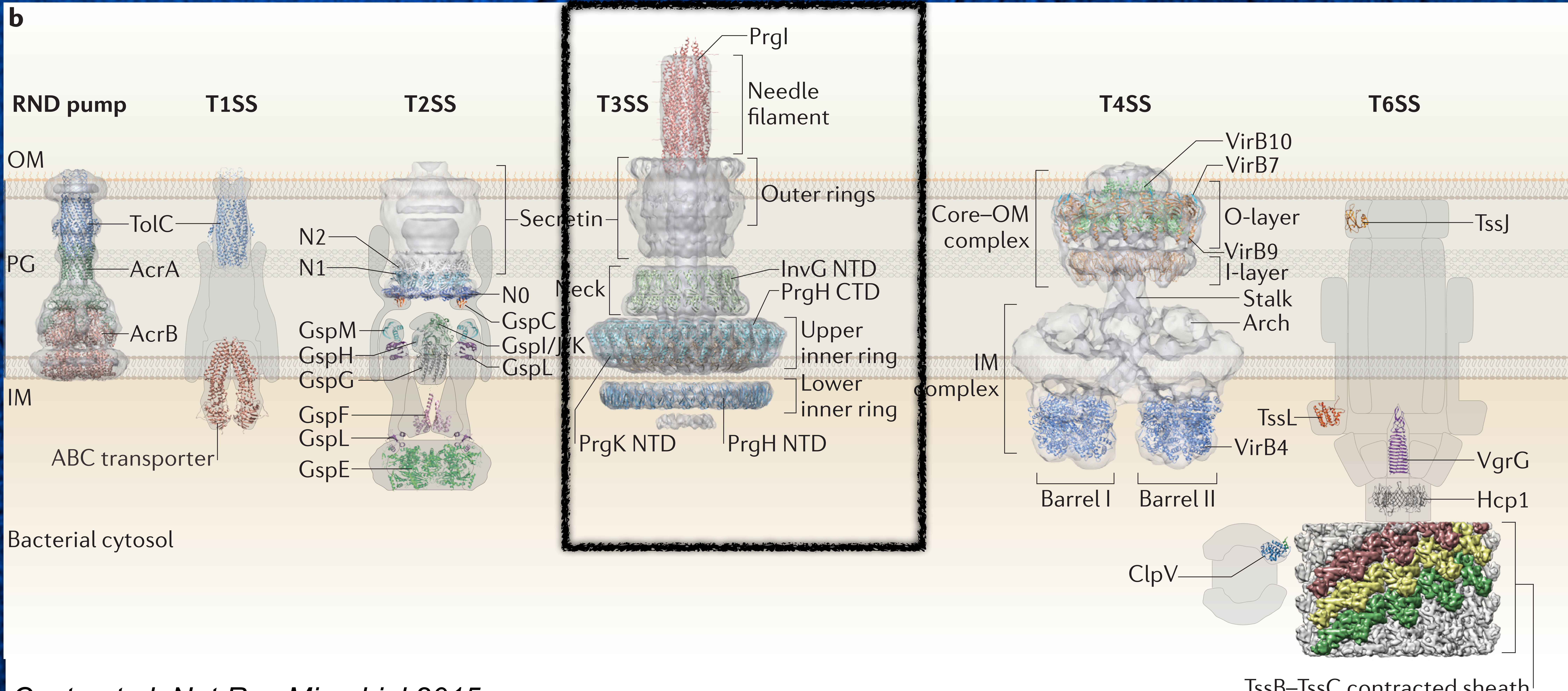
Image from NIAID

Bacterial secretion systems



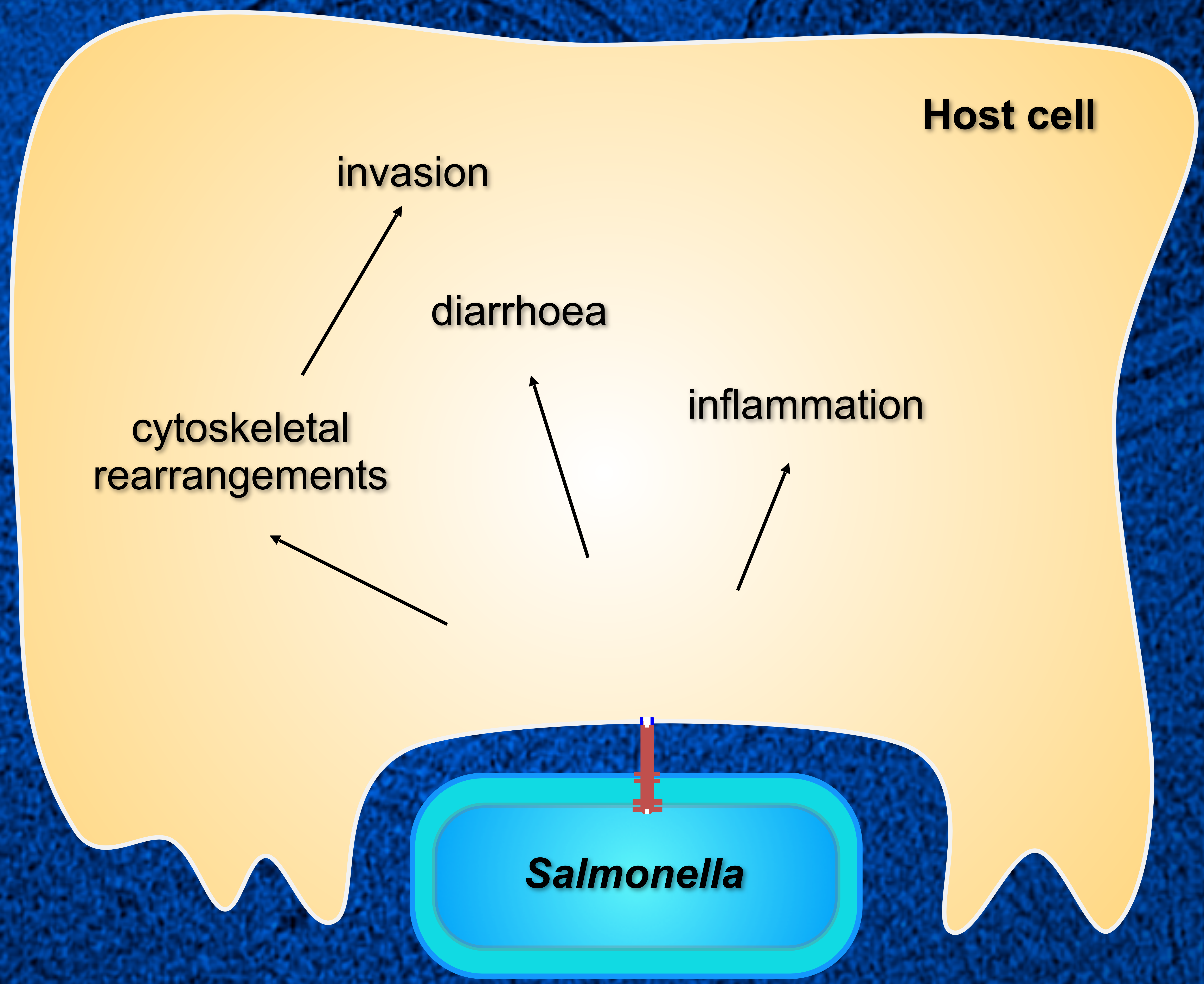
Costa et al, Nat Rev Microbiol 2015

Bacterial secretion systems

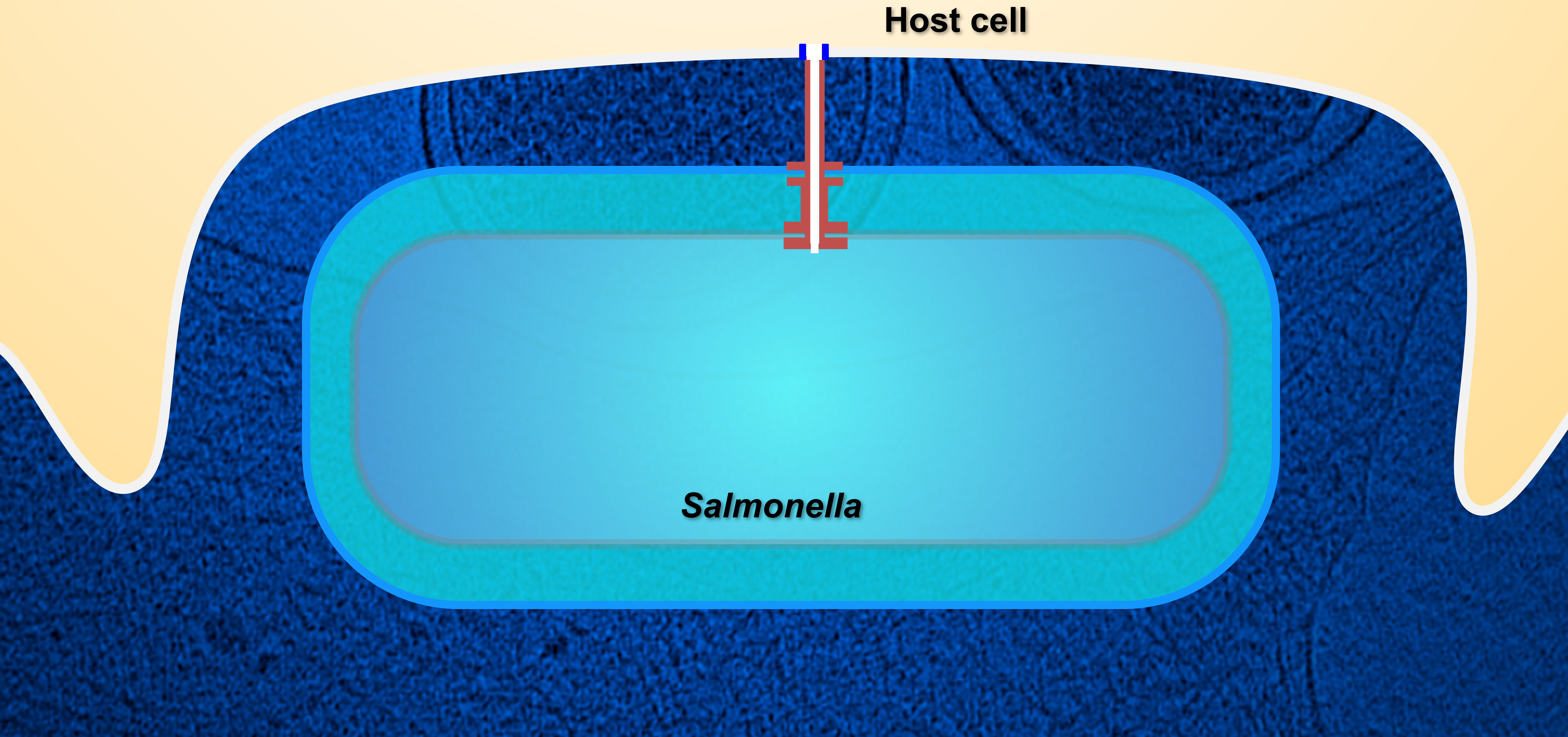


Costa et al, Nat Rev Microbiol 2015

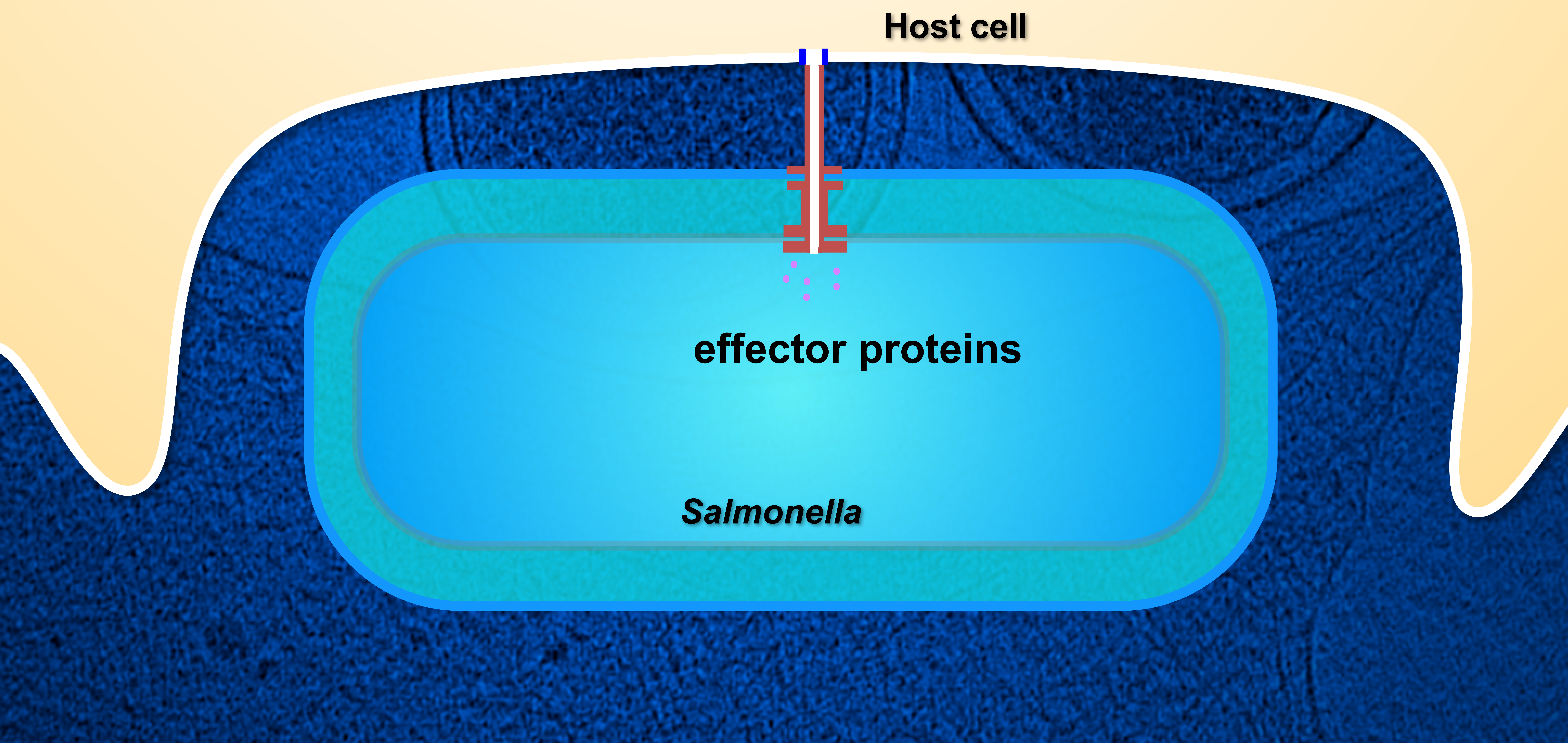
Type III secretion mediated infection



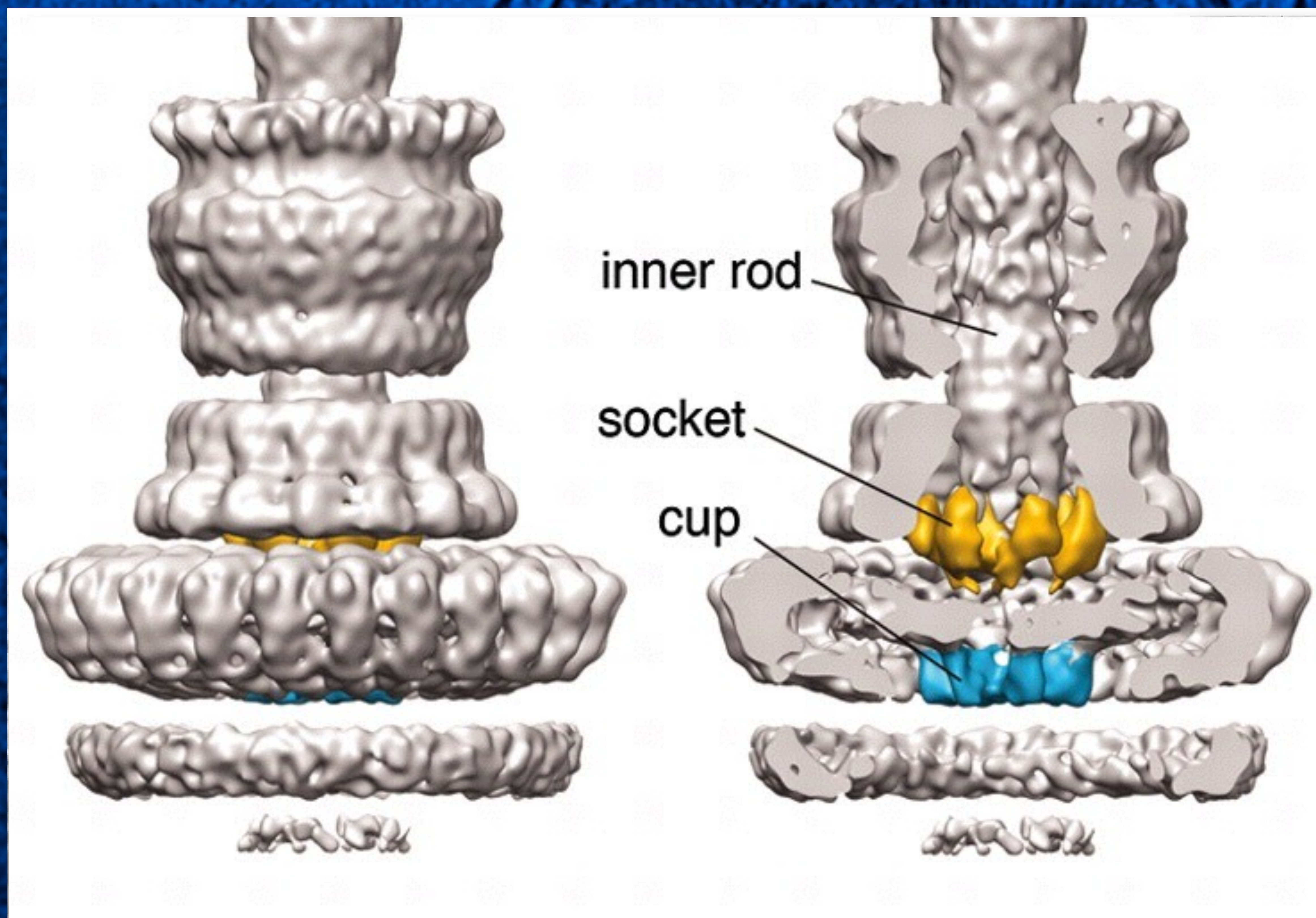
Type III secretion mediated infection



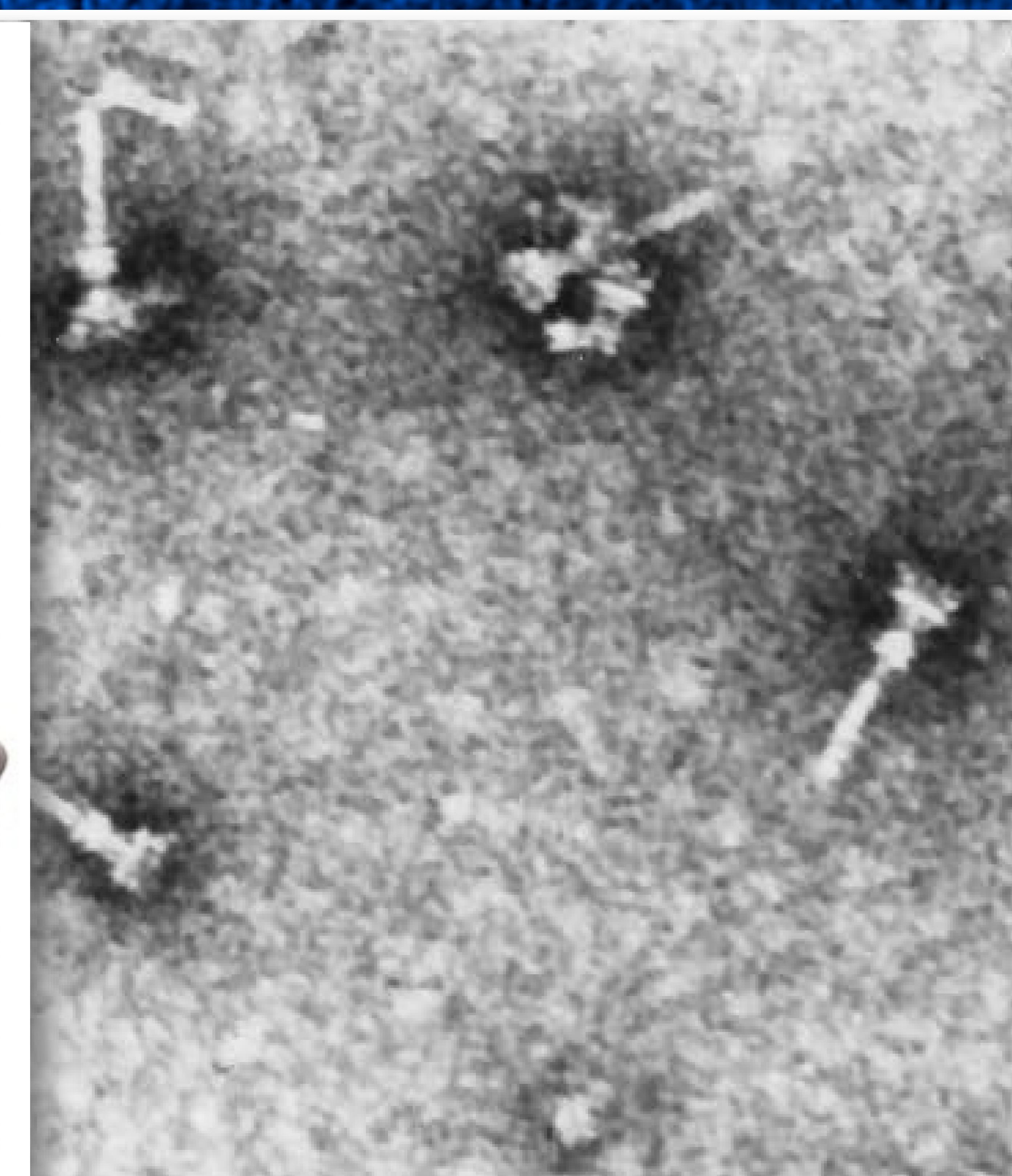
Secretion of bacterial effector proteins



Discovery and characterization of *Salmonella* T3SS-1

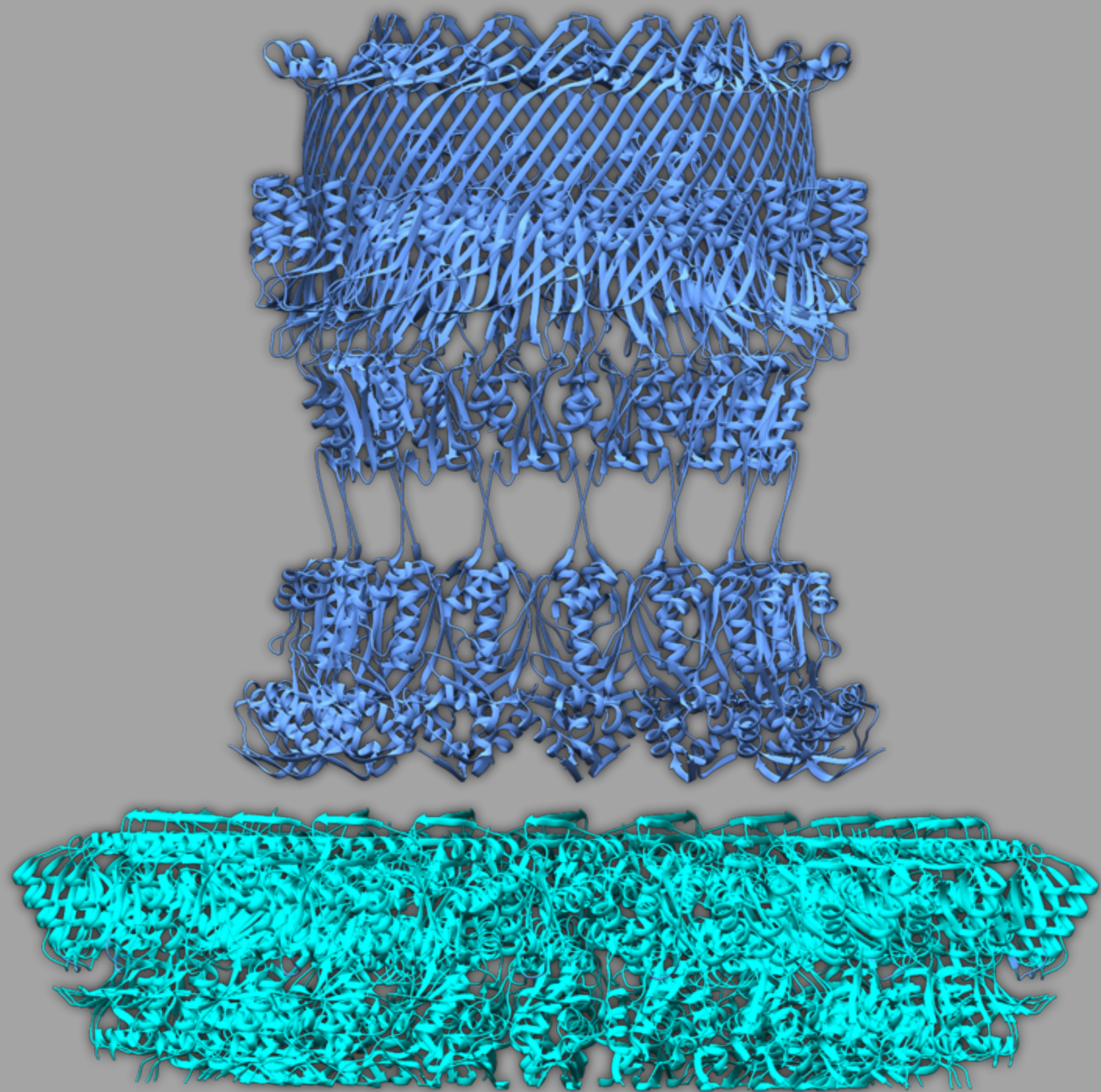


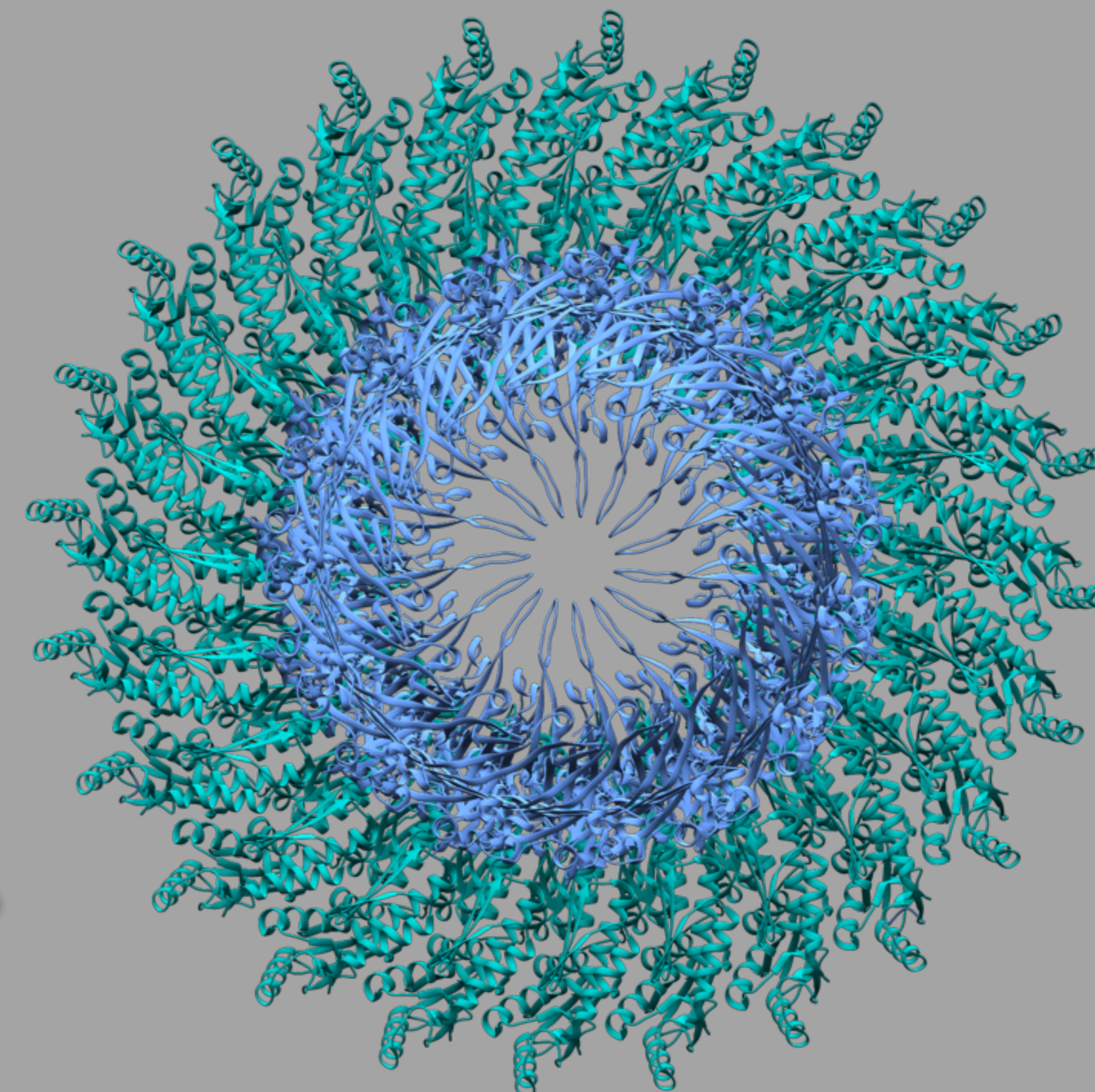
Schraidt & Marlovits Science 2011



Kubori, T. et al. Science 1998

Near-atomic-resolution structure

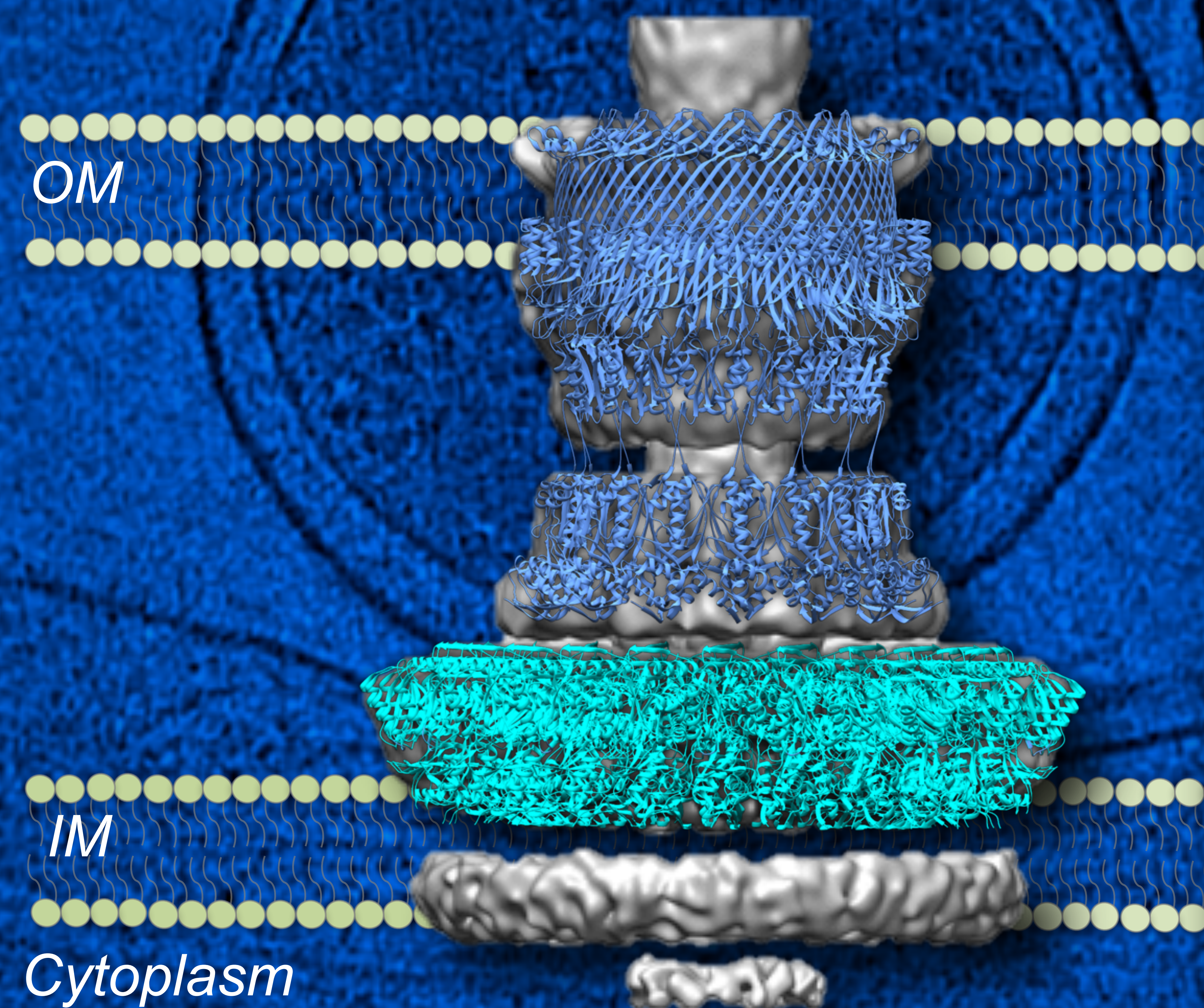




Worrall et al., Nature 2016

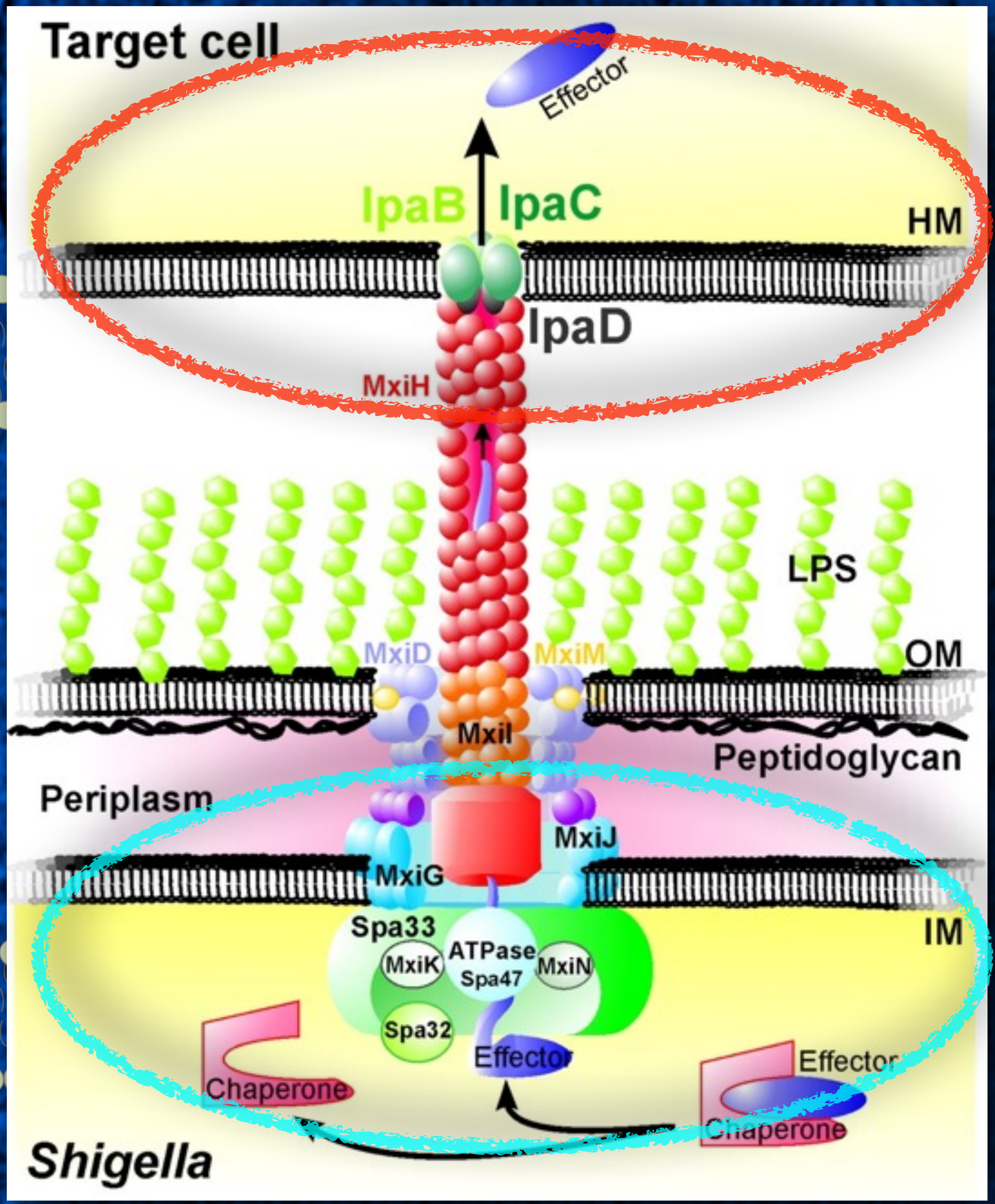
Purified complex lacks key components

Salmonella



Worrall et al., Nature 2016

Schraidt & Marlovits Science 2011

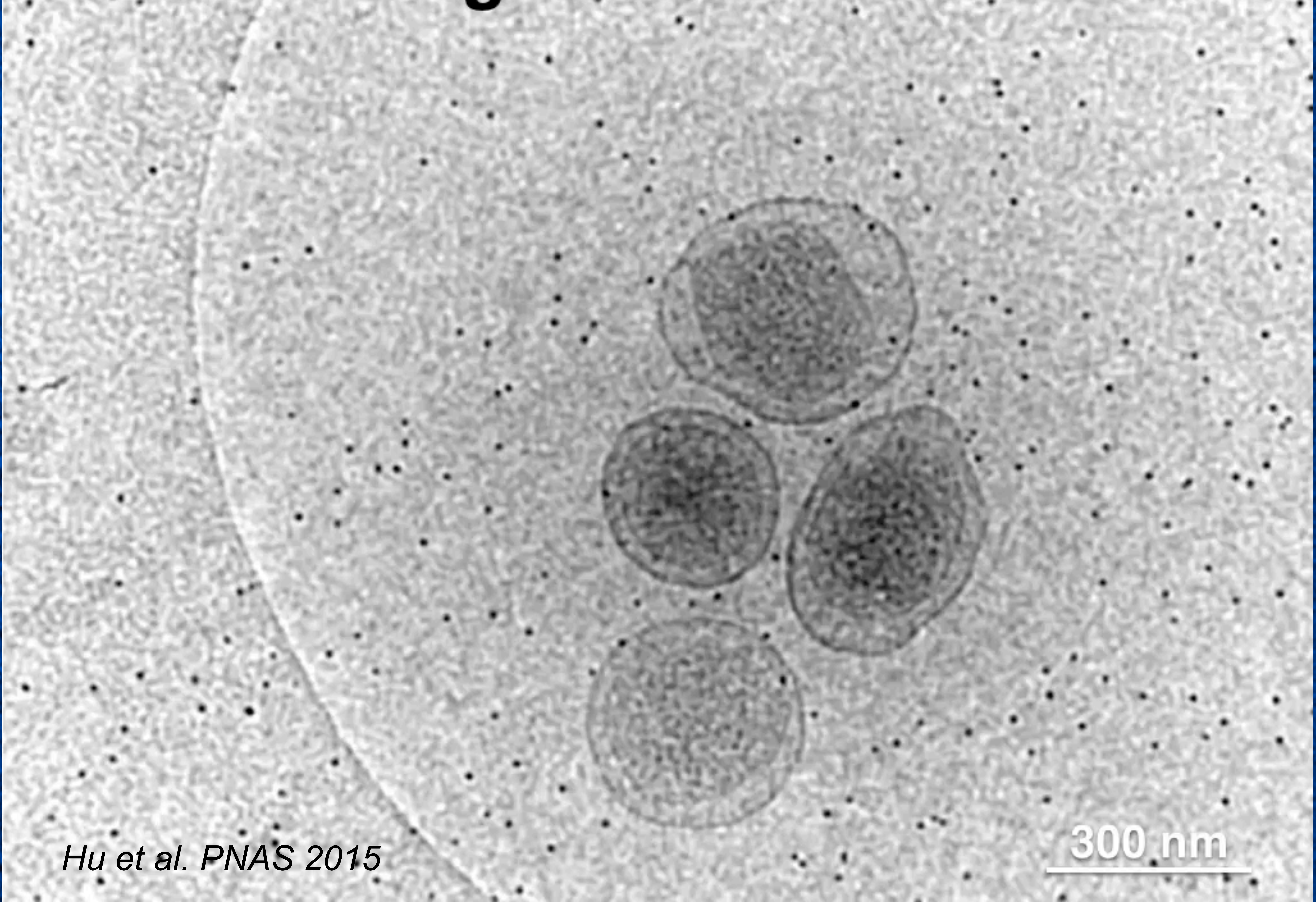


Schroeder & Hilbi 2008

A Sorting Platform Determines the Order of Protein Secretion in Bacterial Type III Systems

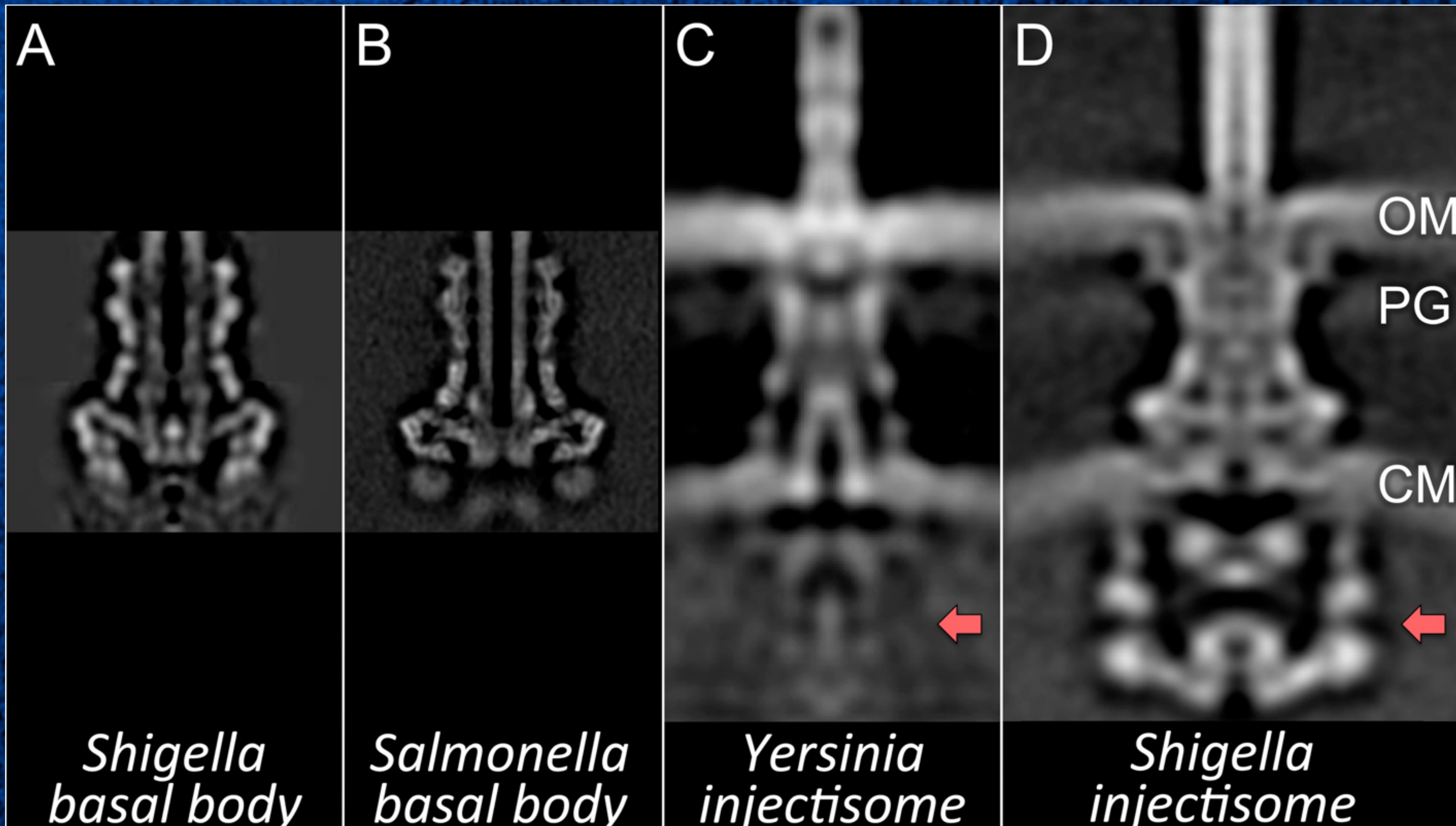
Lara-Tejero et al. ***SCIENCE*** (2011)

Shigella minicells



Hu et al. PNAS 2015

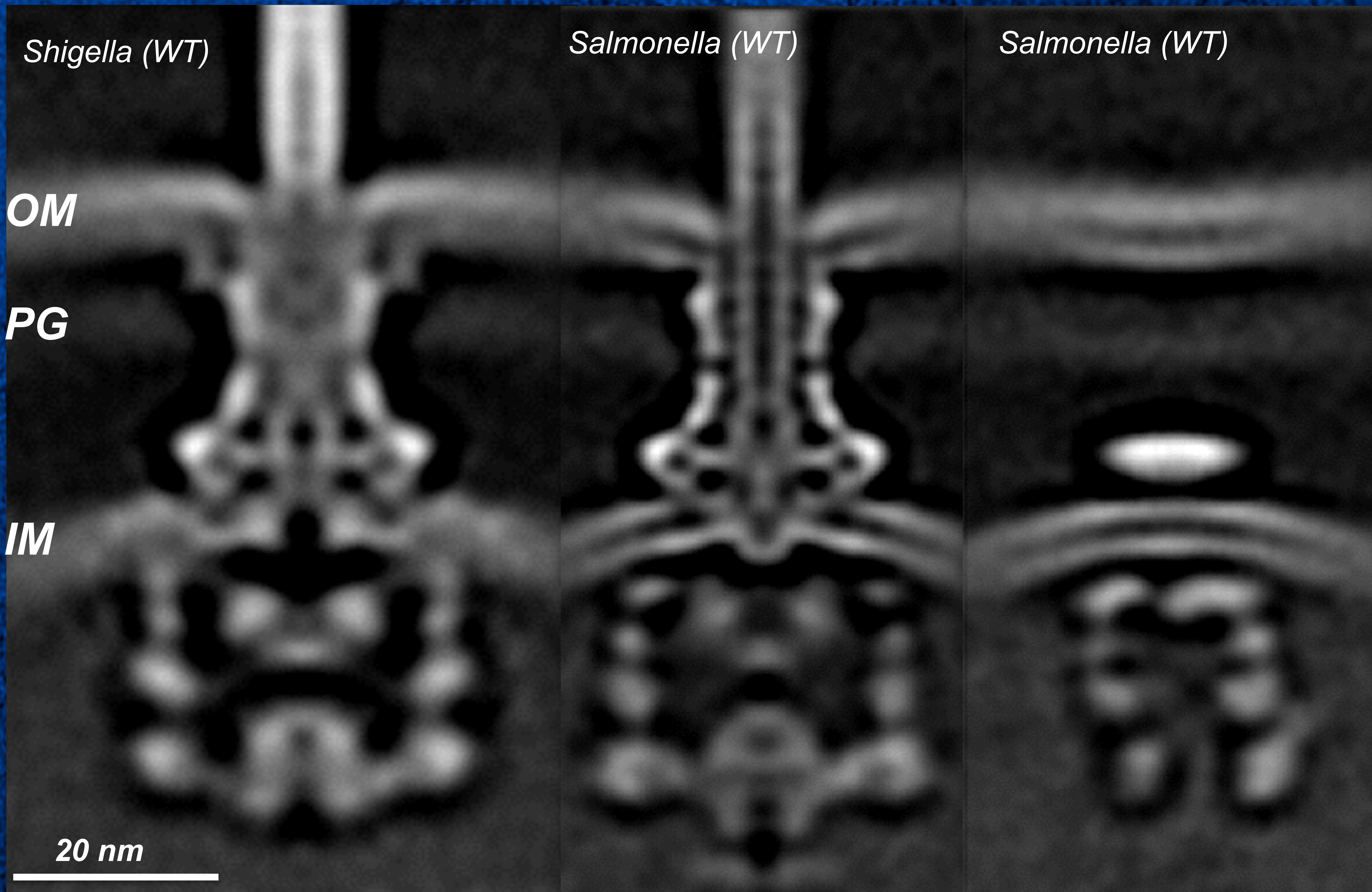
Intact T3SS revealed in *Shigella*



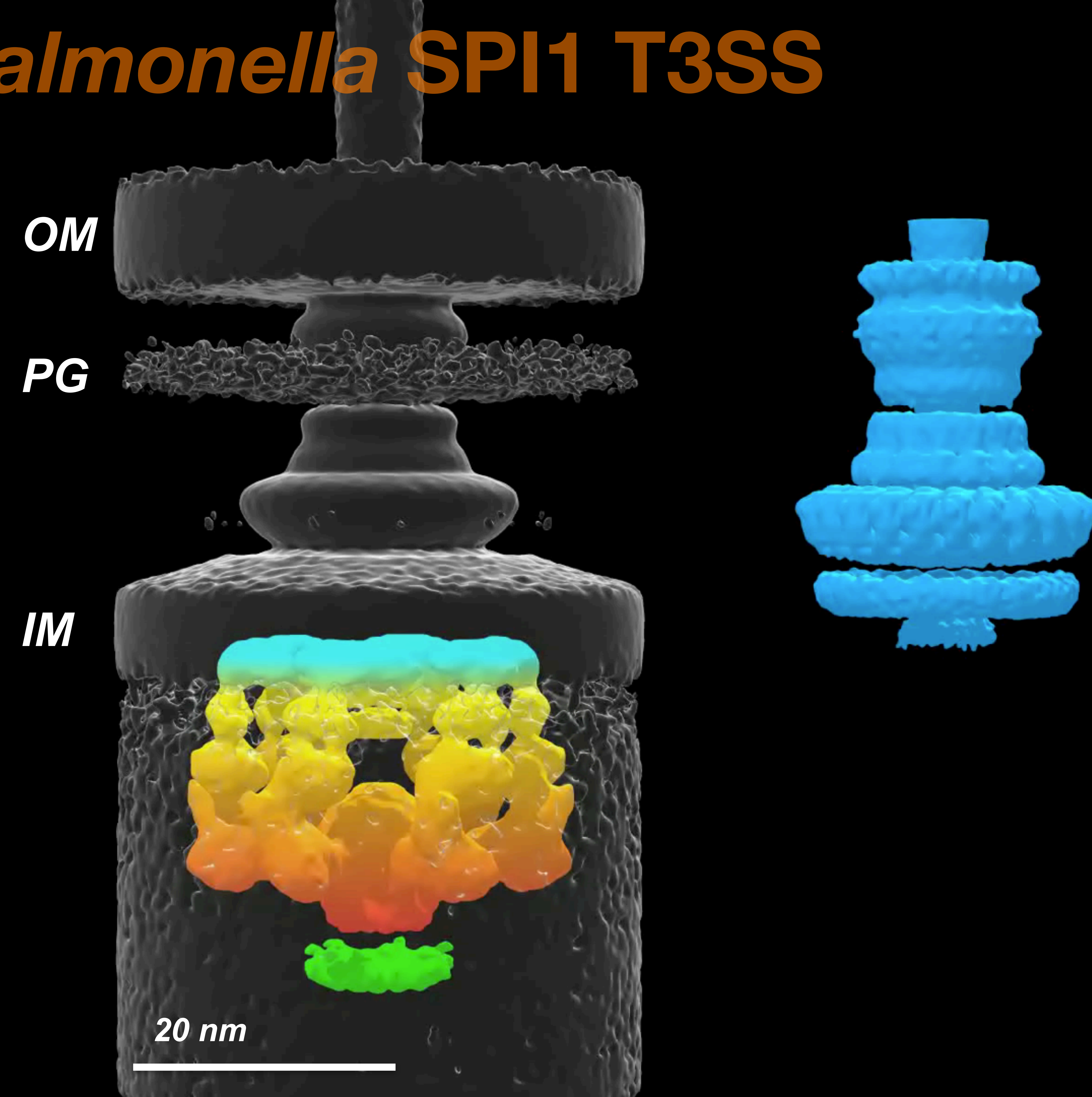
Hodgkinson et al 2009, Schraidt et al. 2011, Kudryashev et al. 2013

Hu et al. PNAS 2015

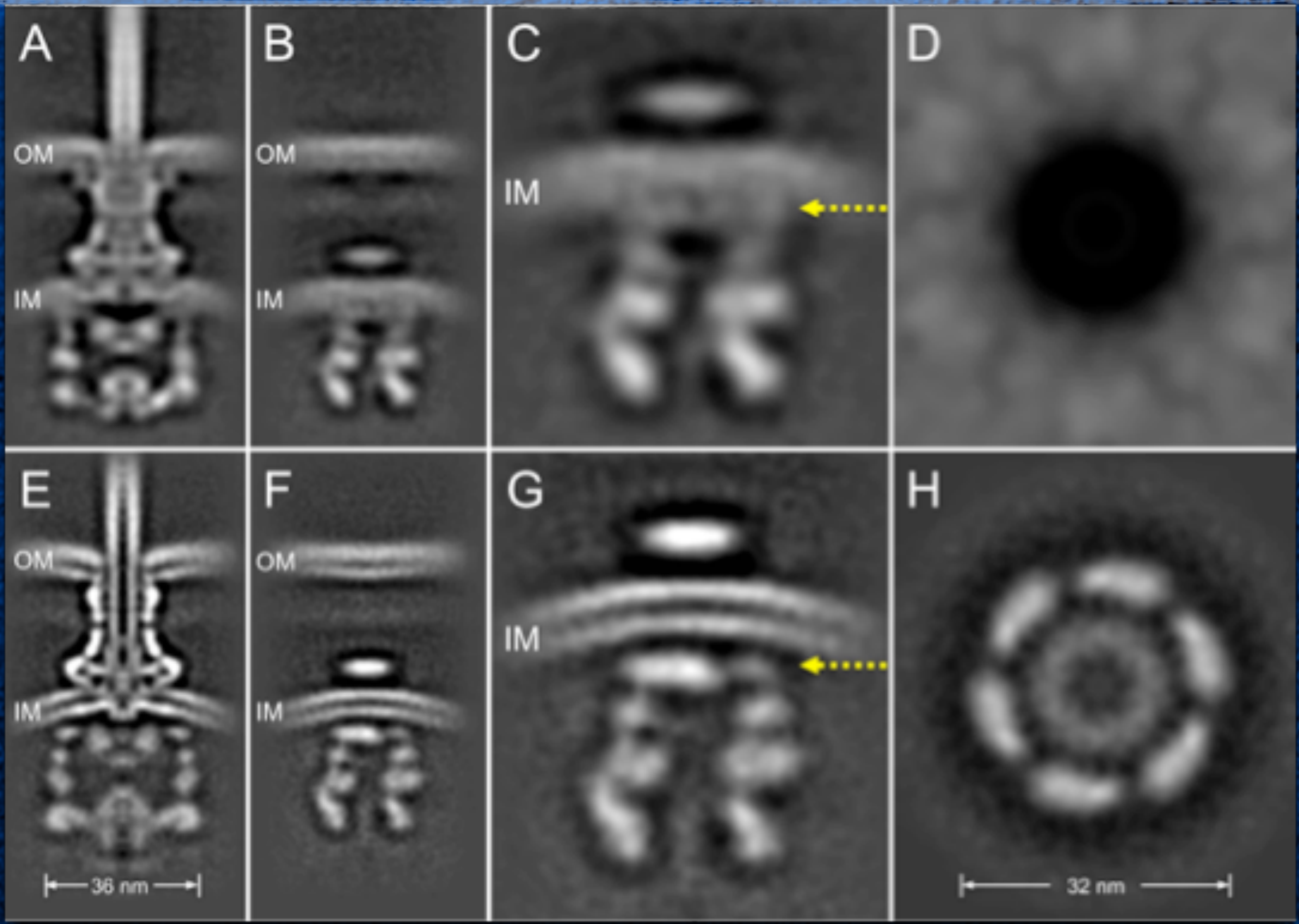
Intact T3SS machine revealed in *Salmonella*



Salmonella SPI1 T3SS



Difference between *Salmonella* and *Shigella*

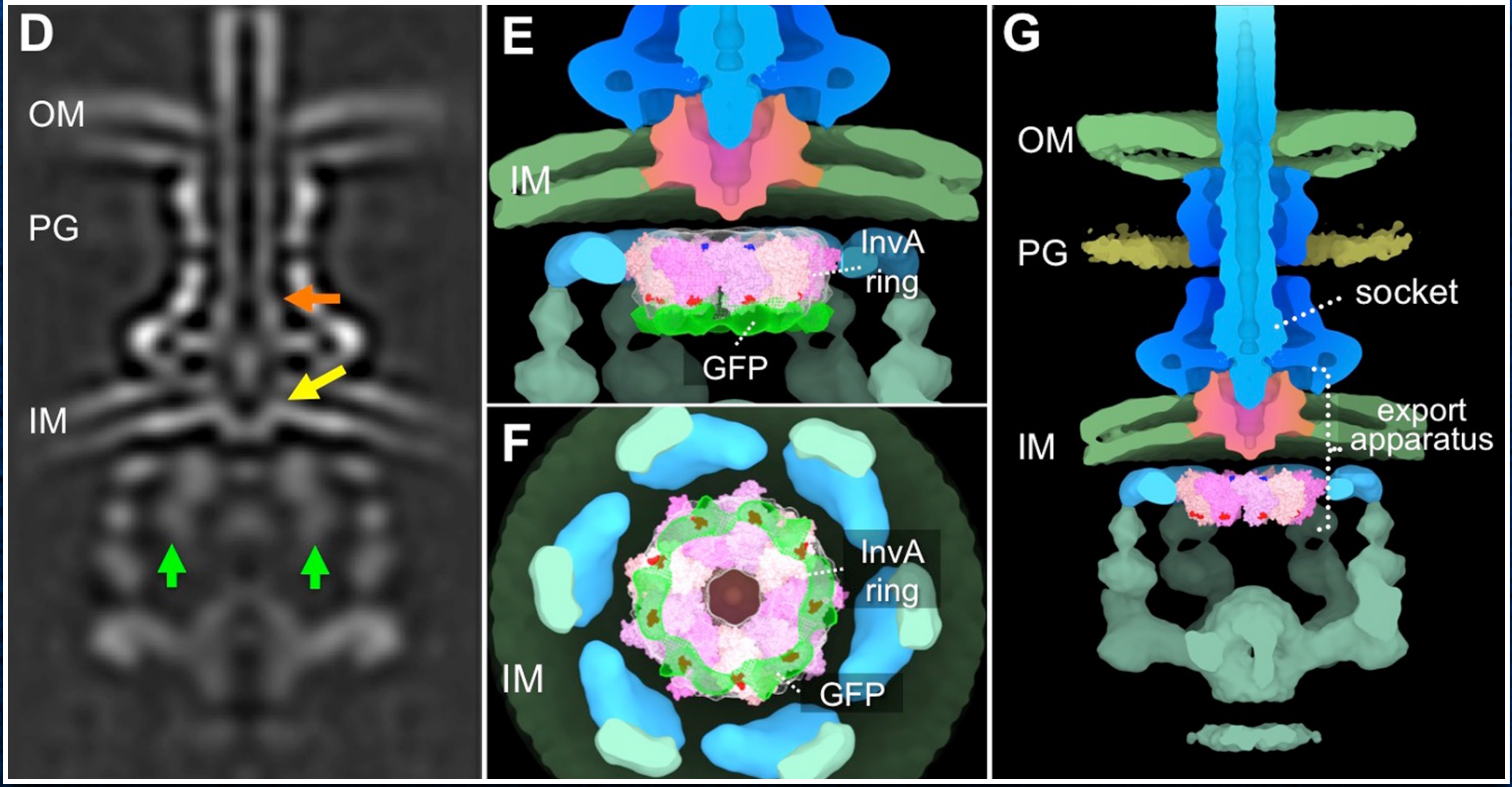


Shigella

Salmonella

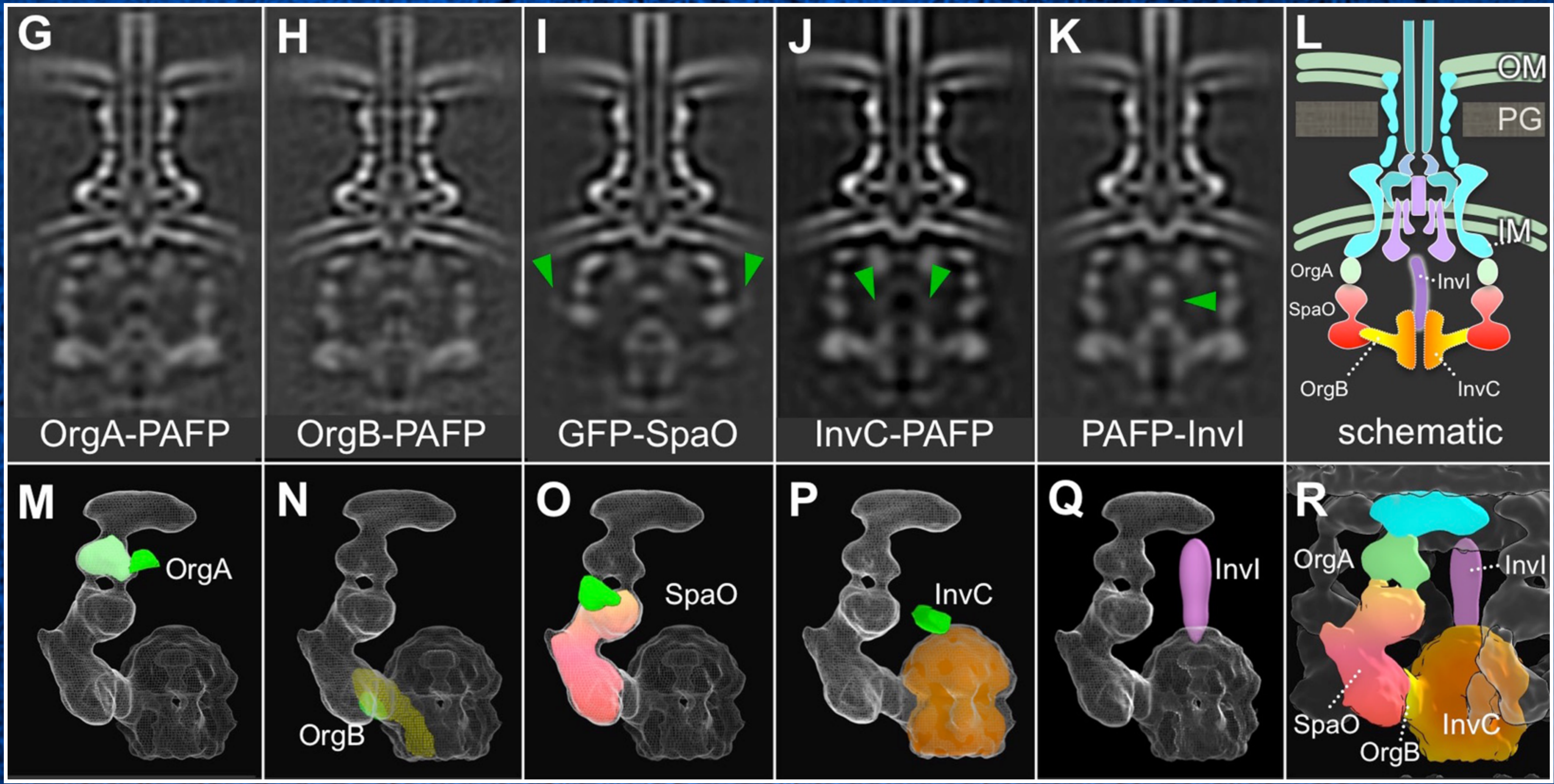
Hu et al. PNAS 2015
Hu et al. Cell 2017

Molecular architecture of the export apparatus



Hu et al. Cell 2017

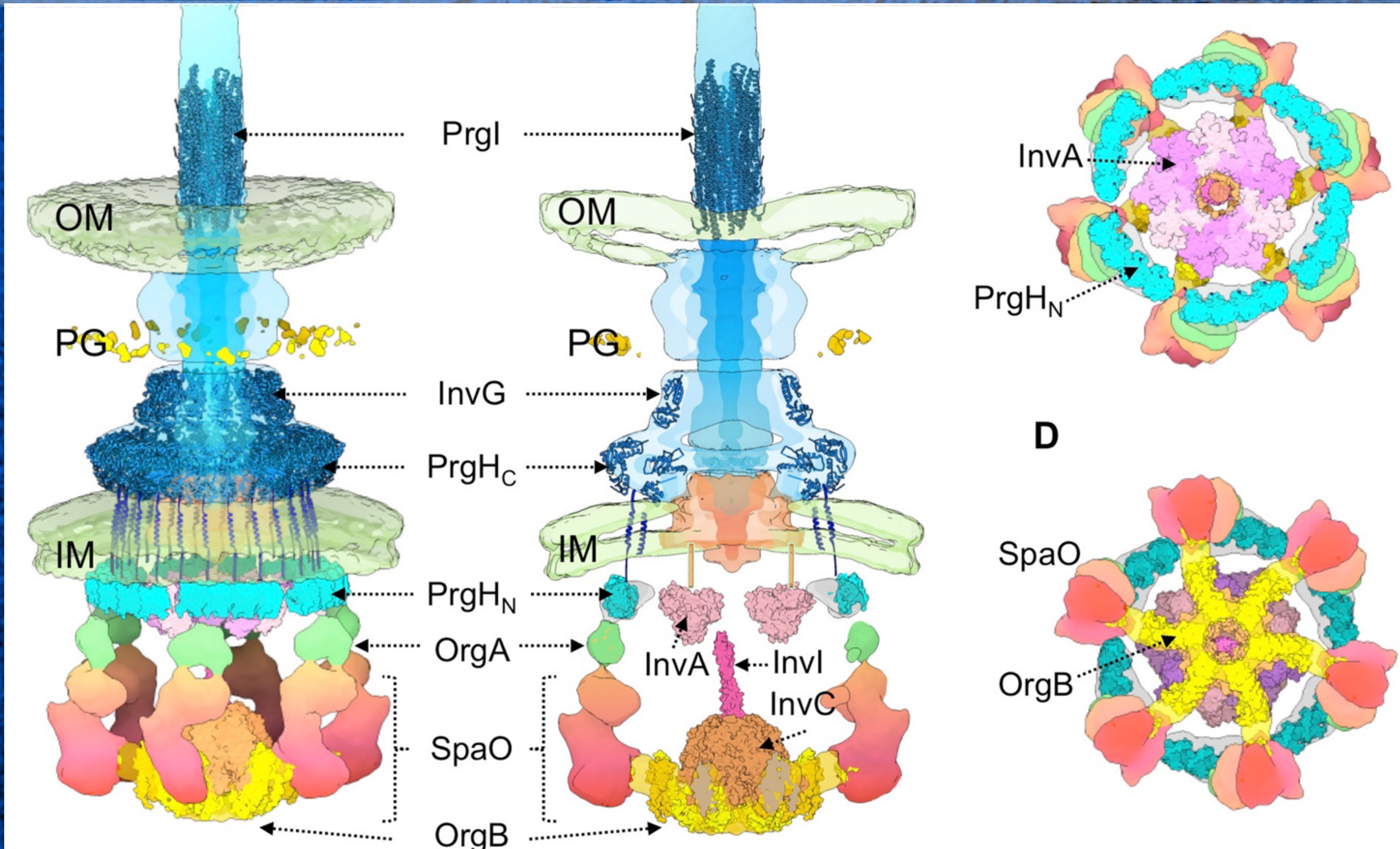
Structural characterization of the sorting platform



Hu et al. Cell 2017

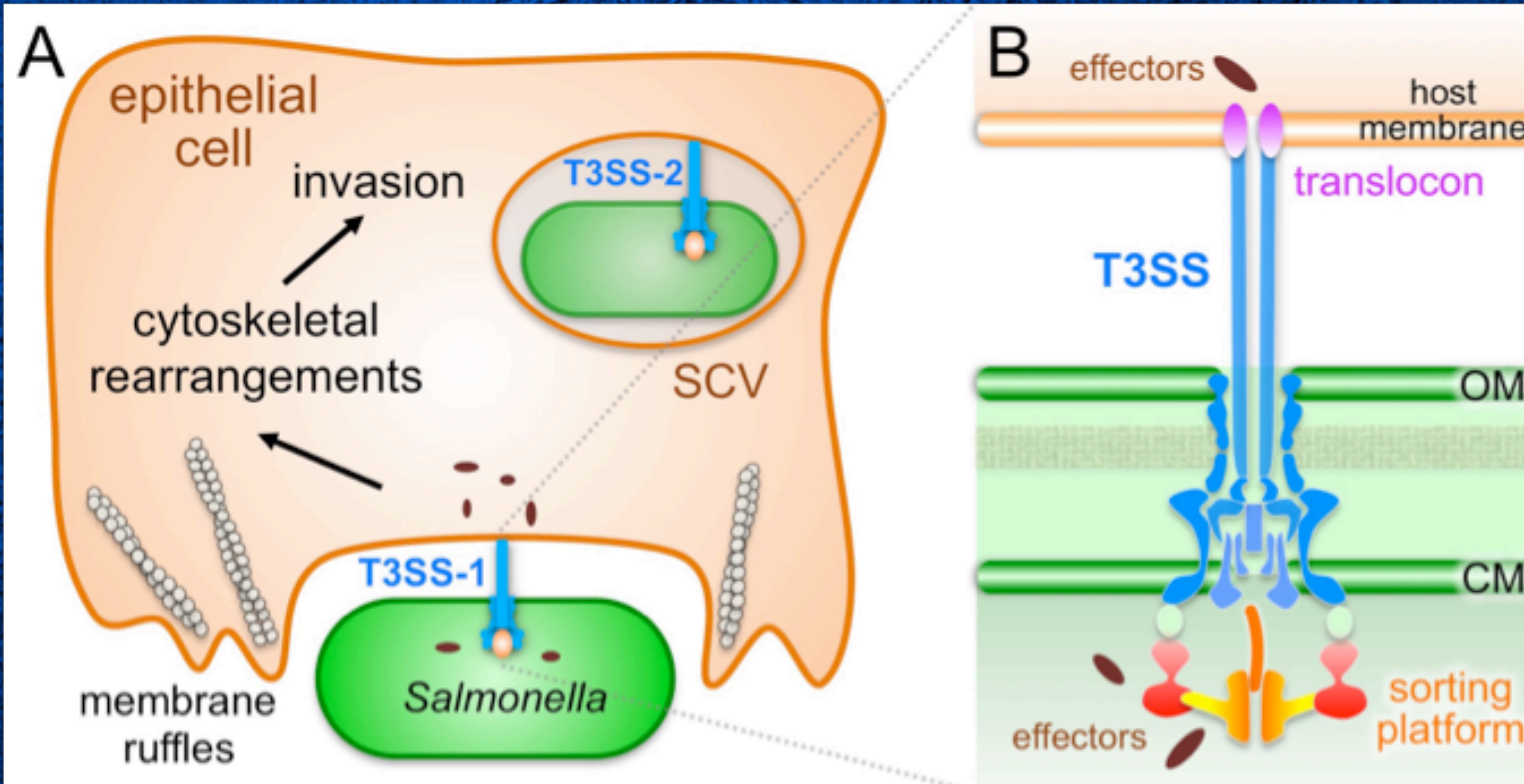
GFP tags on key components

Molecular architecture of the T3SS machine *in situ*

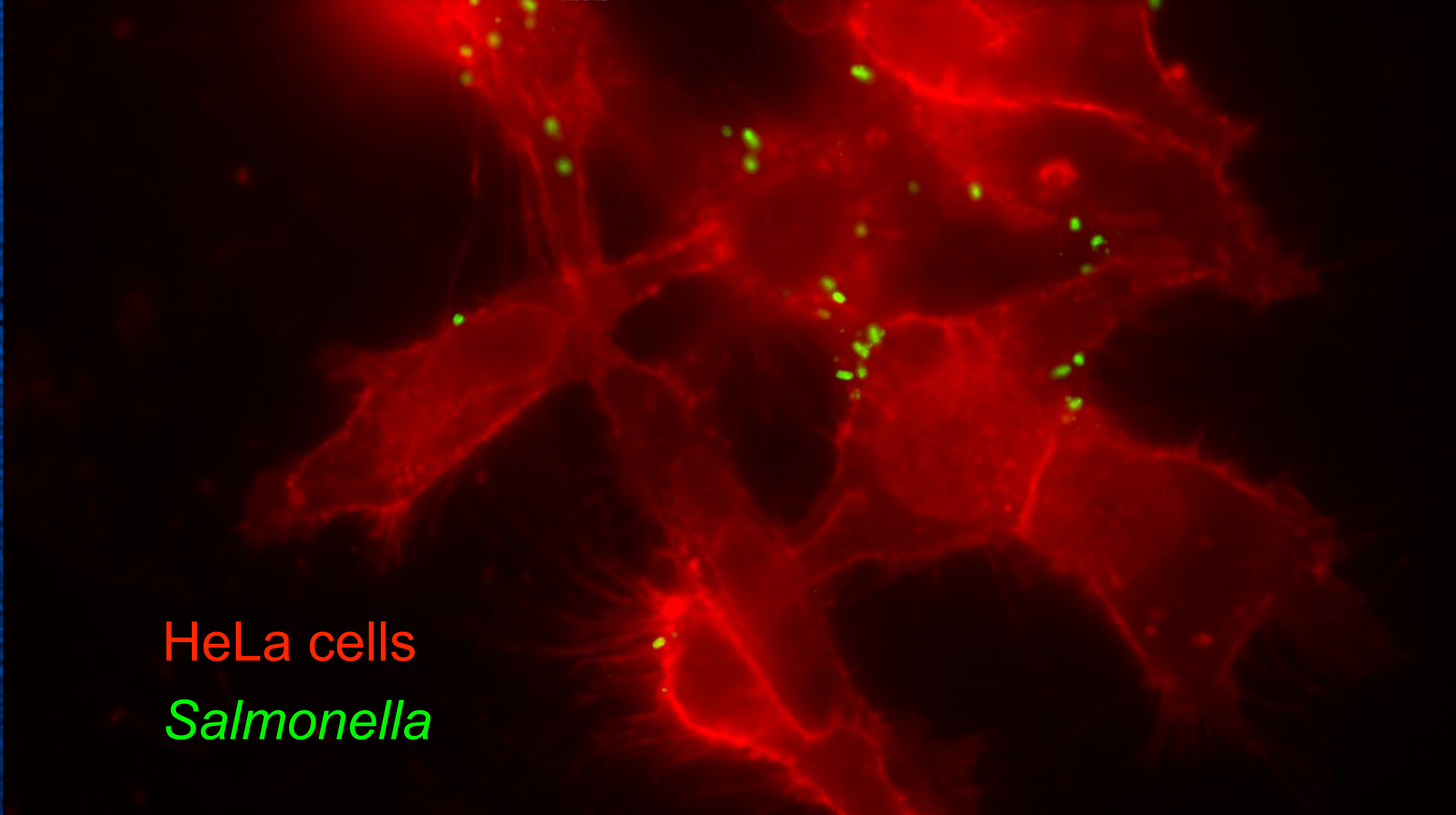


Hu et al. Cell 2017

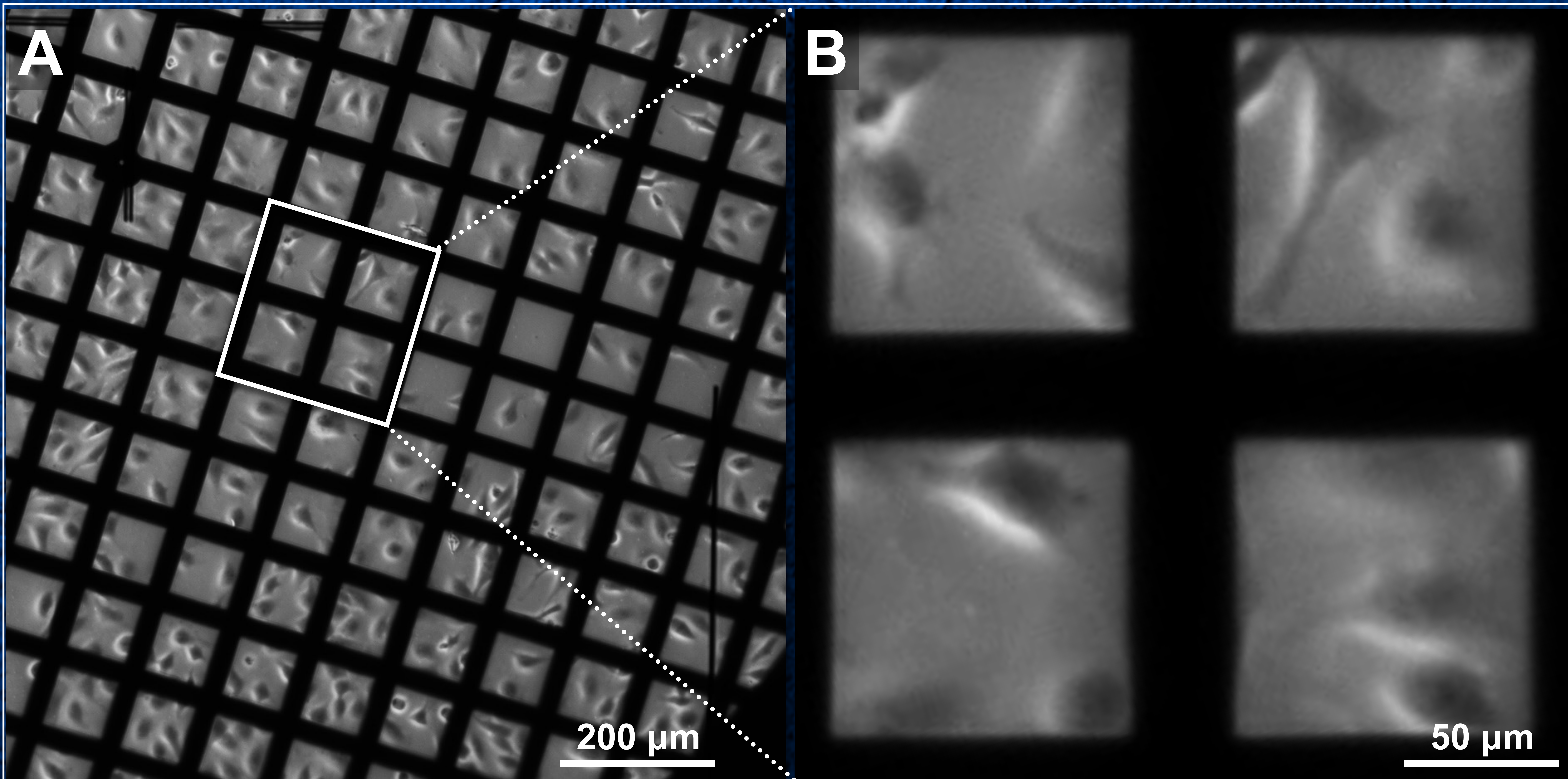
Type III secretion mediated infection



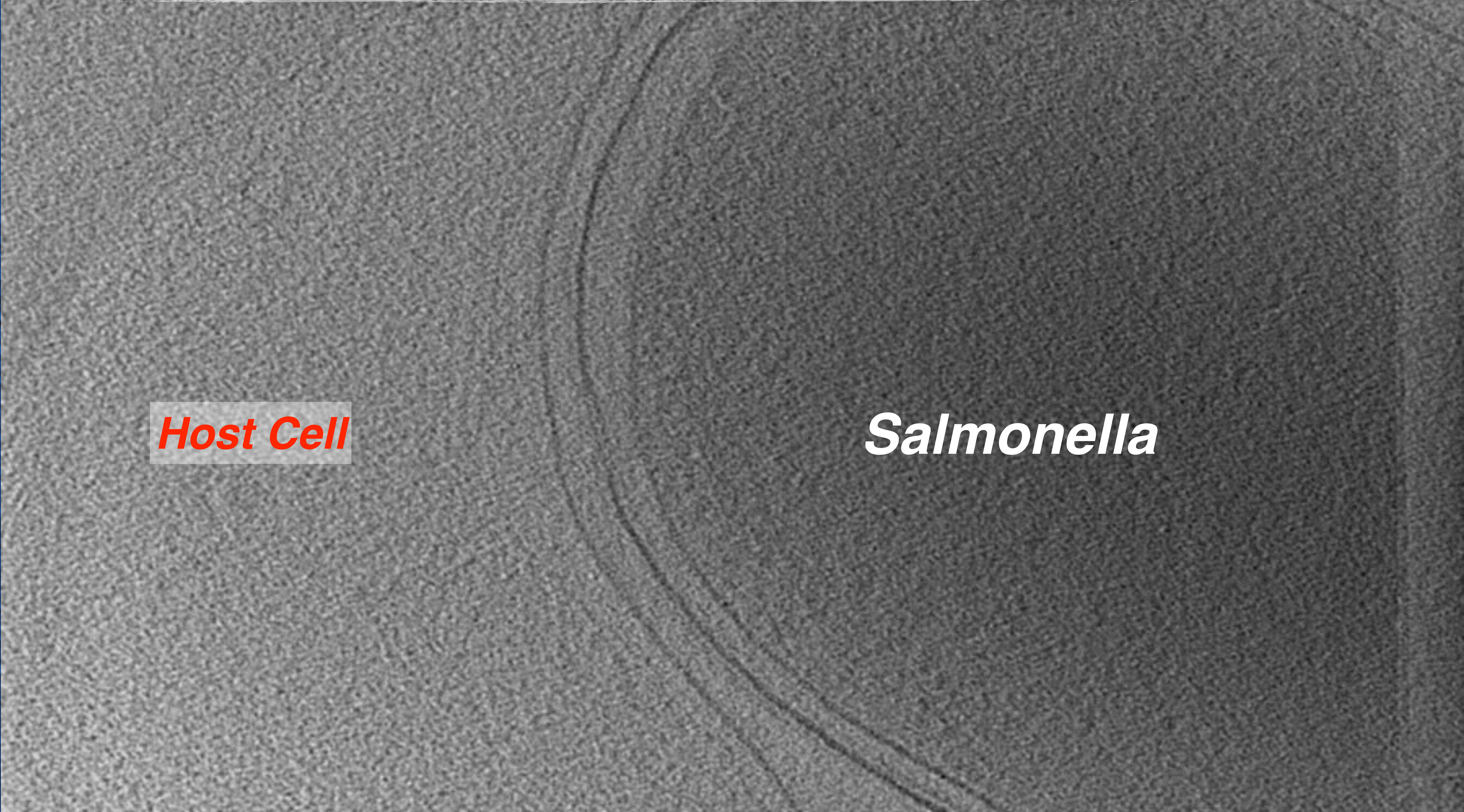
Host cells infected by *Salmonella*

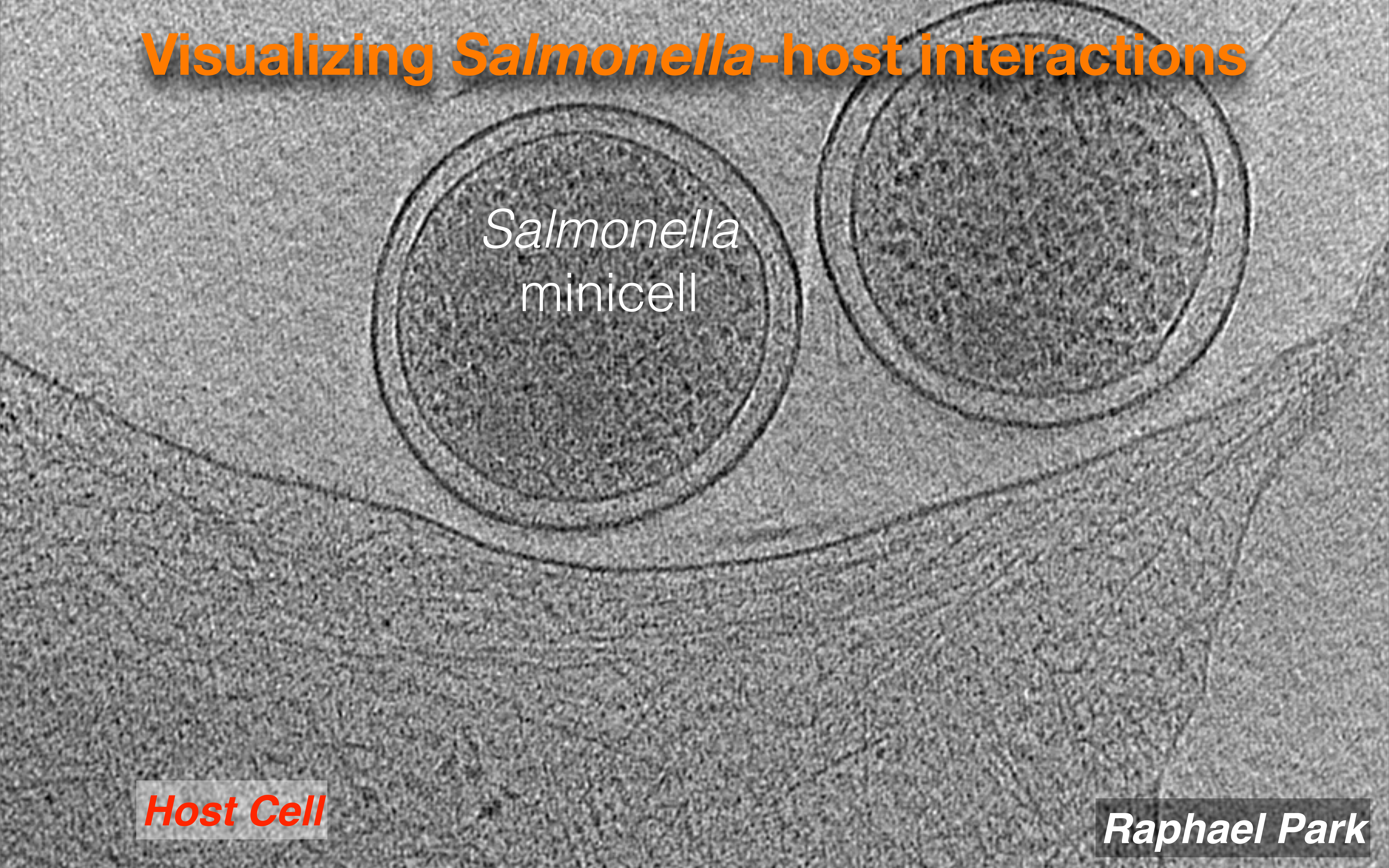


HeLa cells on EM grid



Salmonella-host interaction




Visualizing *Salmonella*-host interactions
Salmonella
minicell
Host Cell
Raphael Park

Bacterial Nonamachines (III)

Phage infection

Novel insights into virus-host interaction and transient channel formation

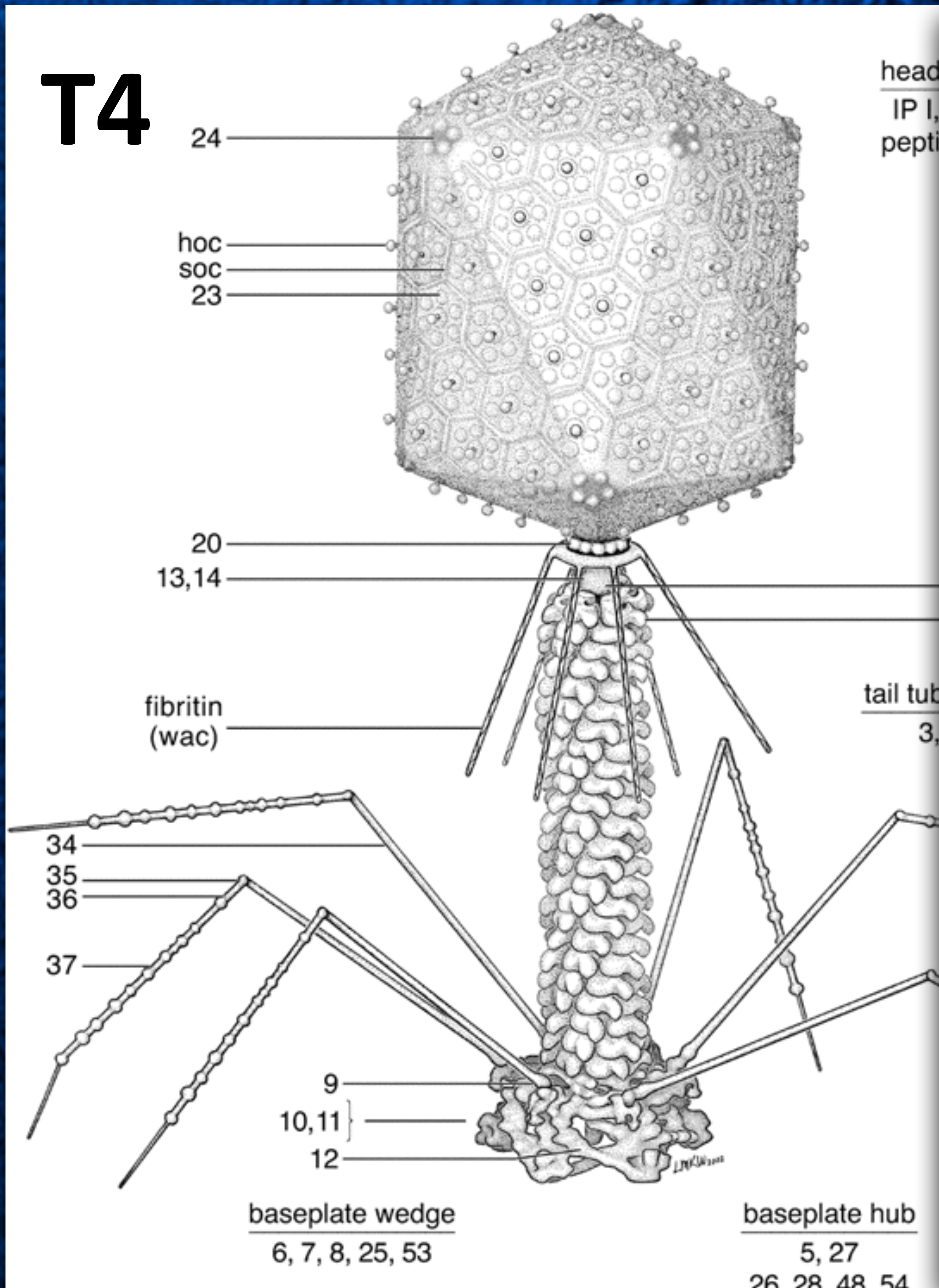
Collaborators:
Dr. Ian Molineux

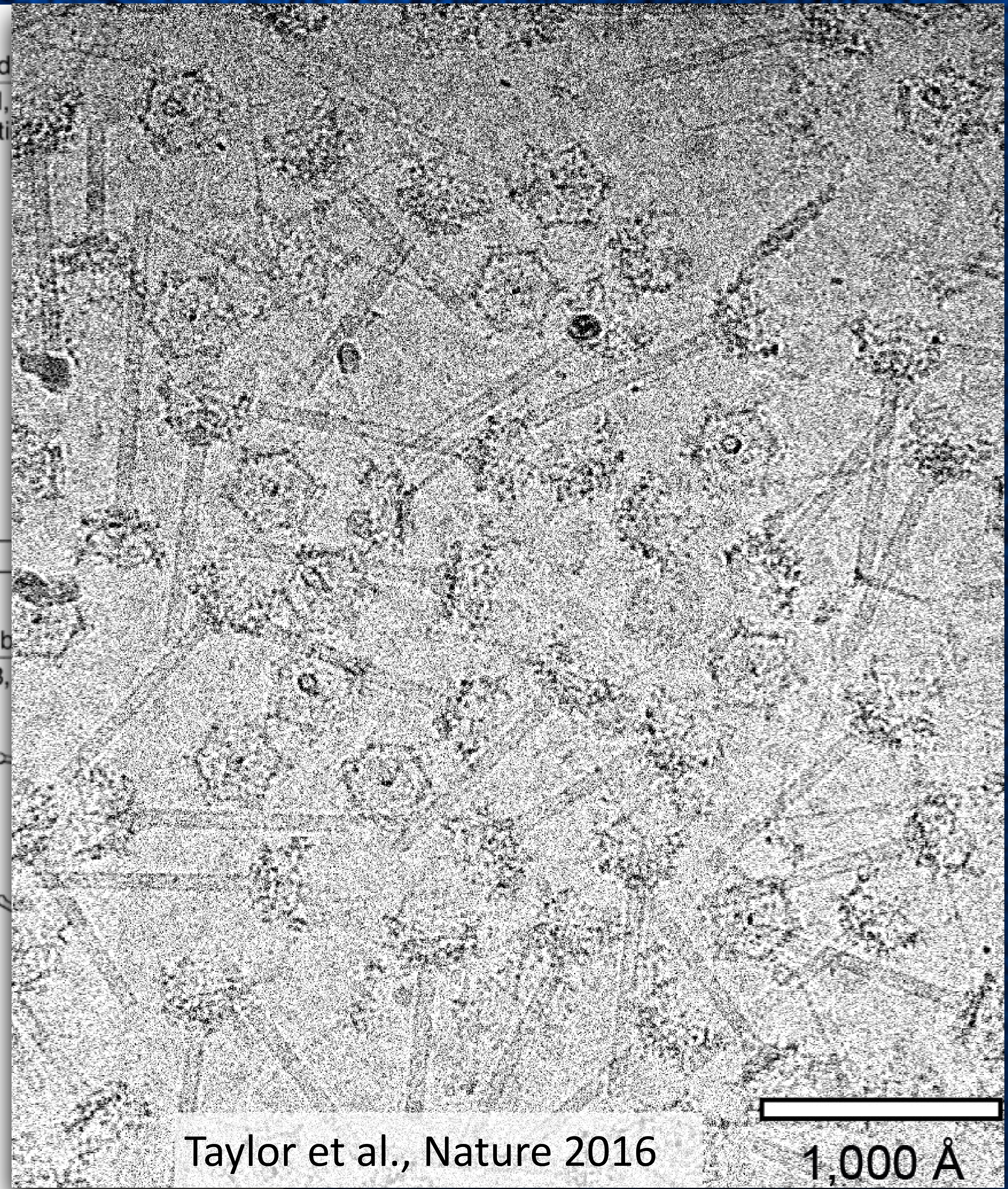
Supported by NIH/NIGMS R01GM110243 & R01GM124378

Bacteriophages - Amazing nanomachines

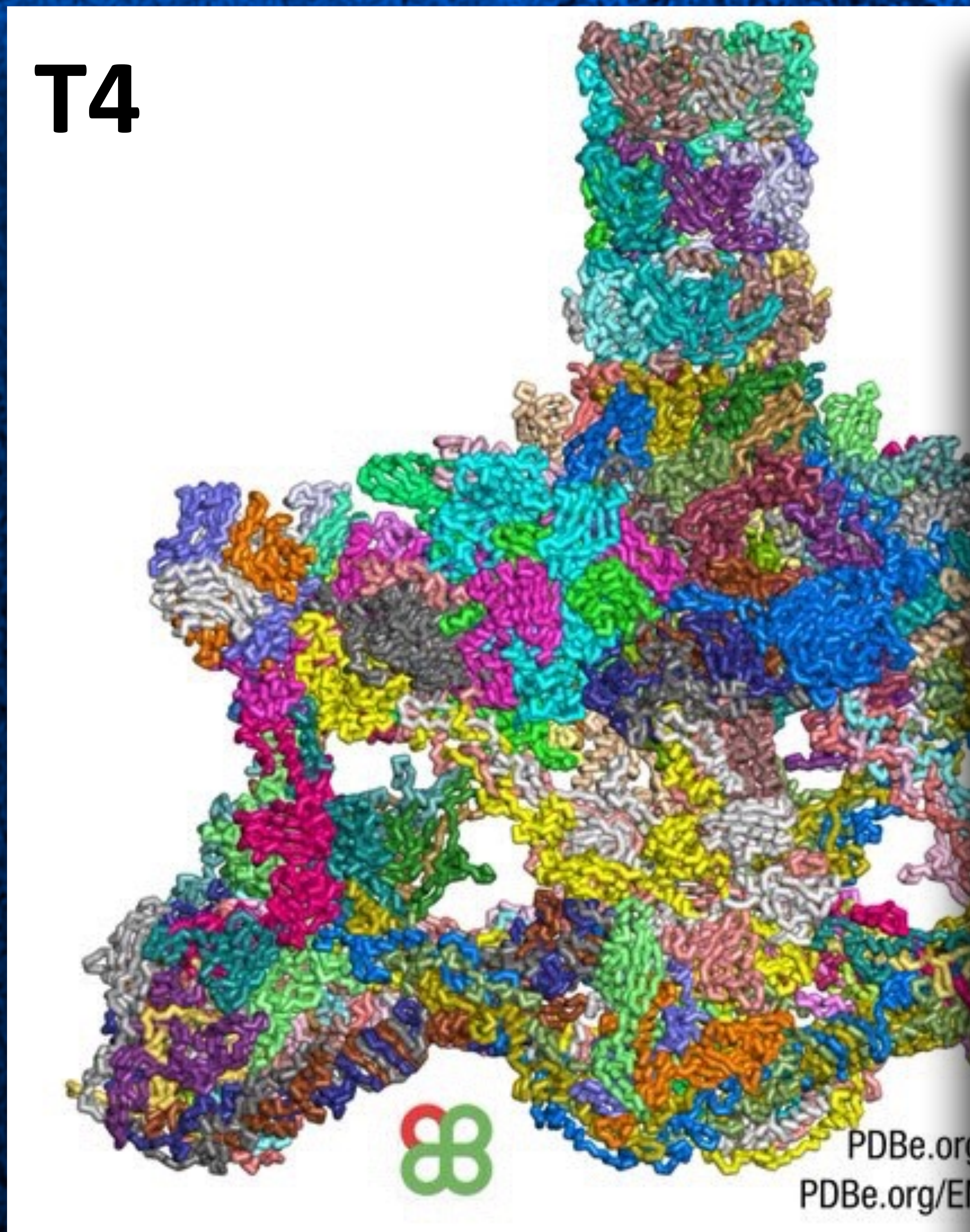
(Kleinschmidt et al., 1962)

Bacteriophages - Amazing nanomachines



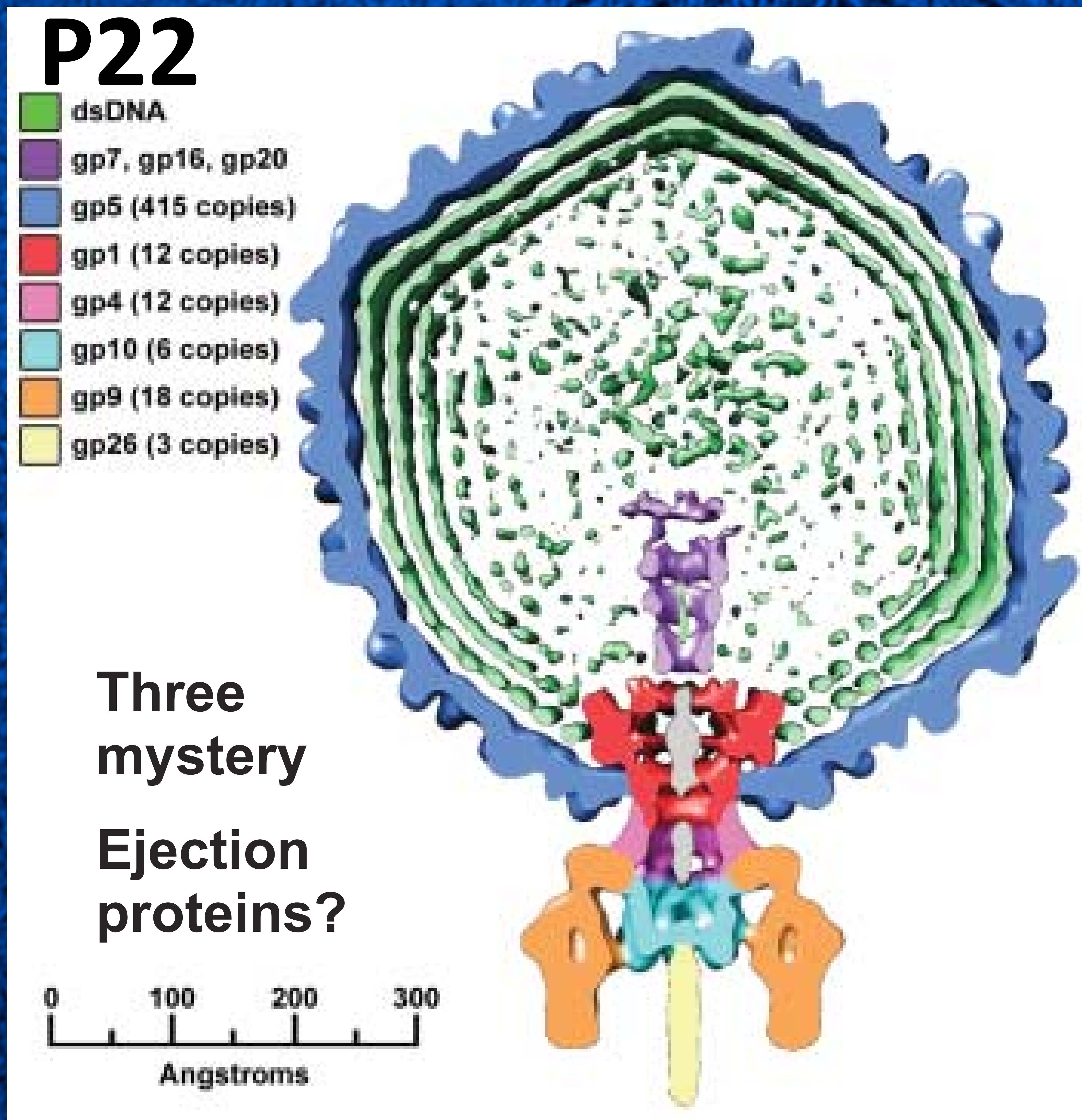


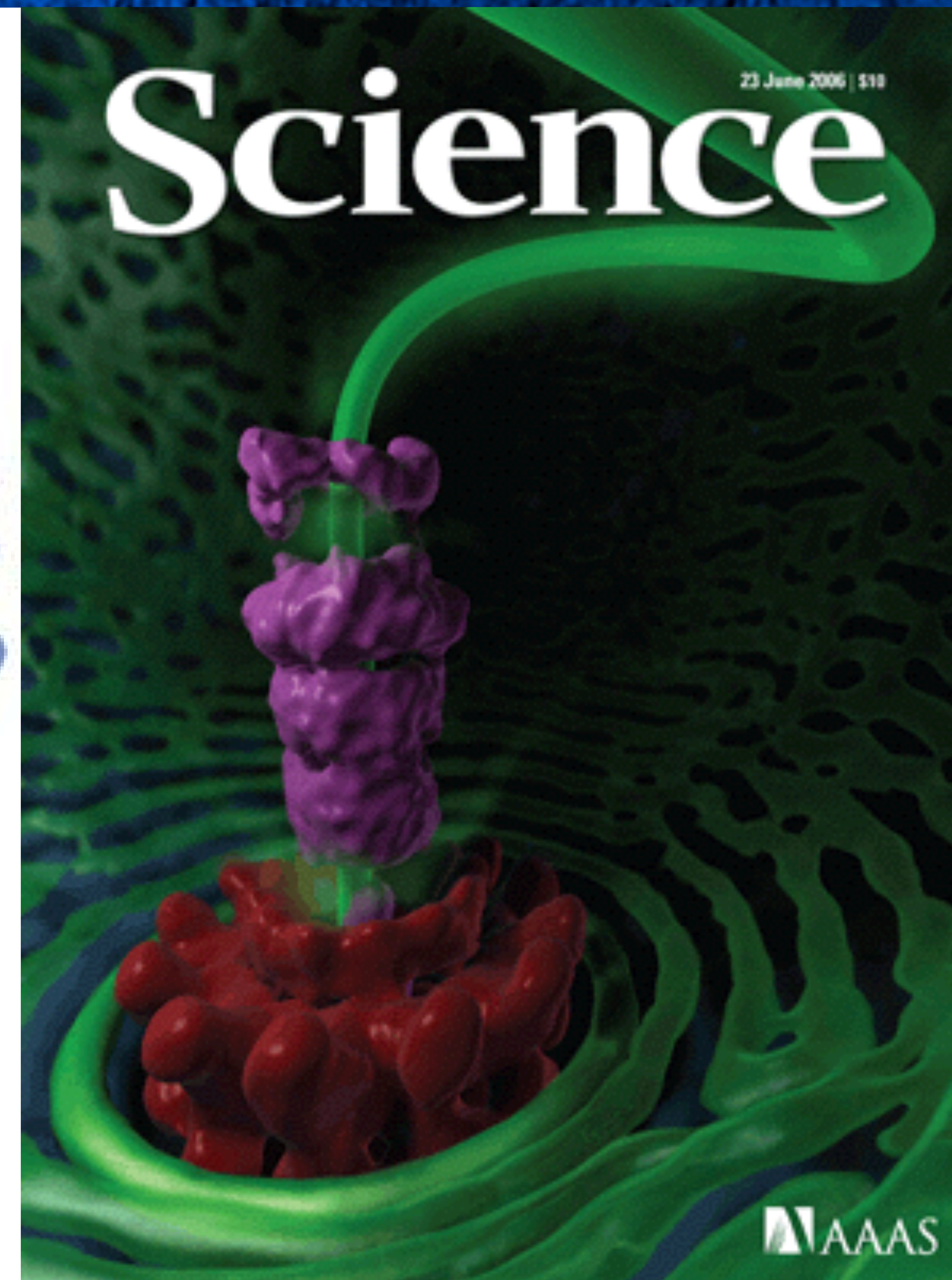
Bacteriophages - Amazing nanomachines



Taylor et al., Nature 2016

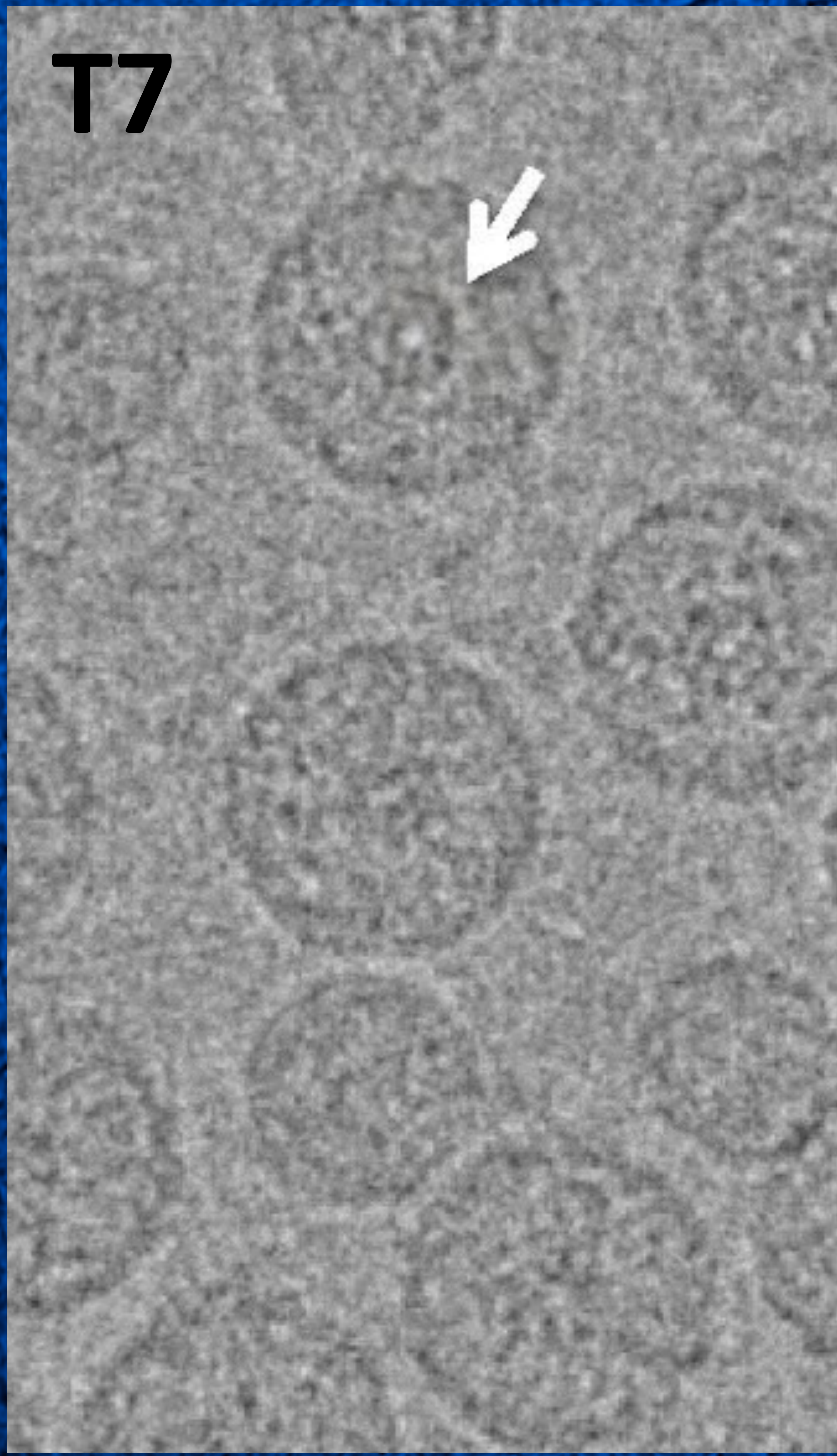
Bacteriophages - Amazing nanomachines

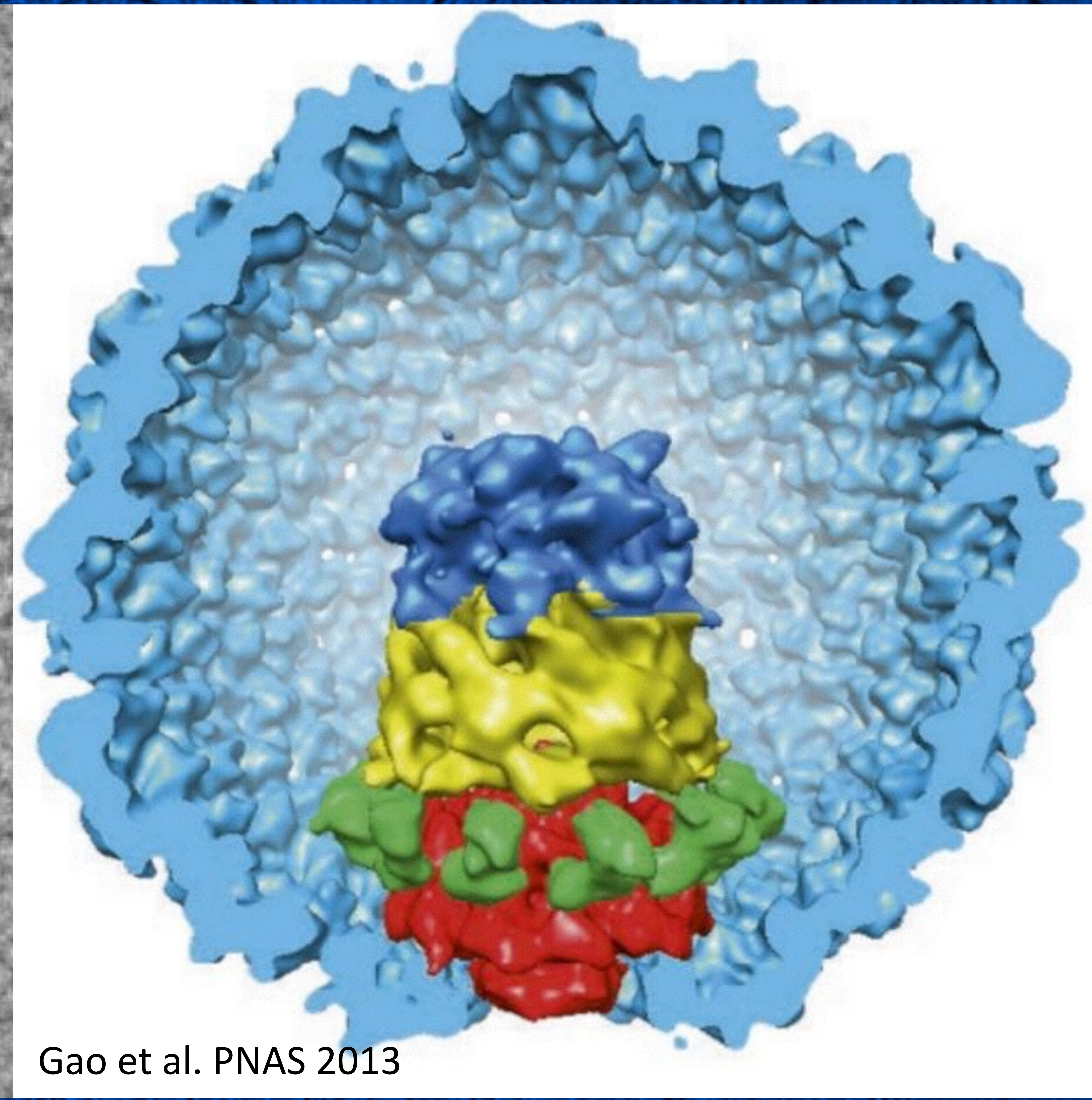




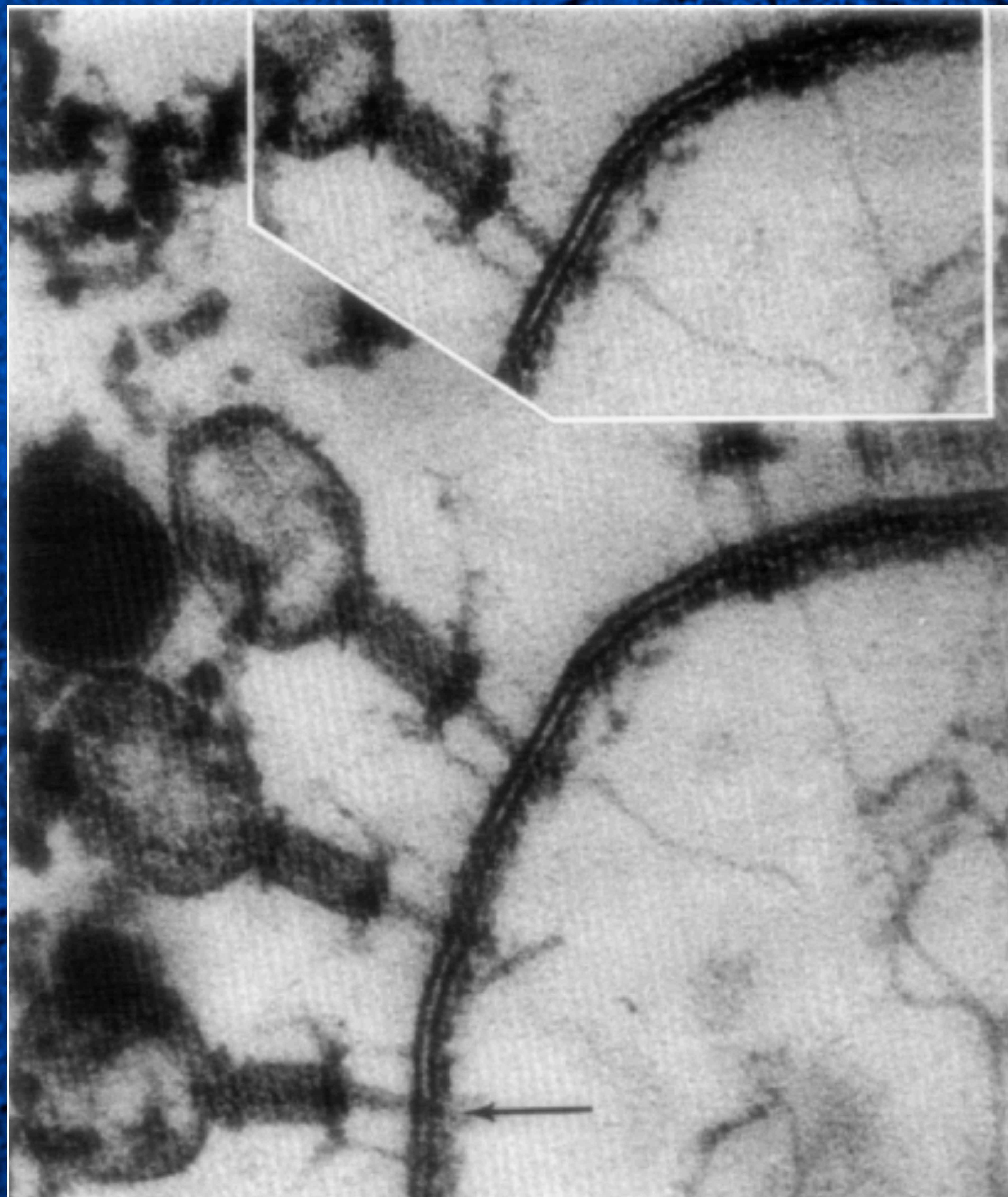
Lander et al. Science 2006

Bacteriophages - Amazing nanomachines

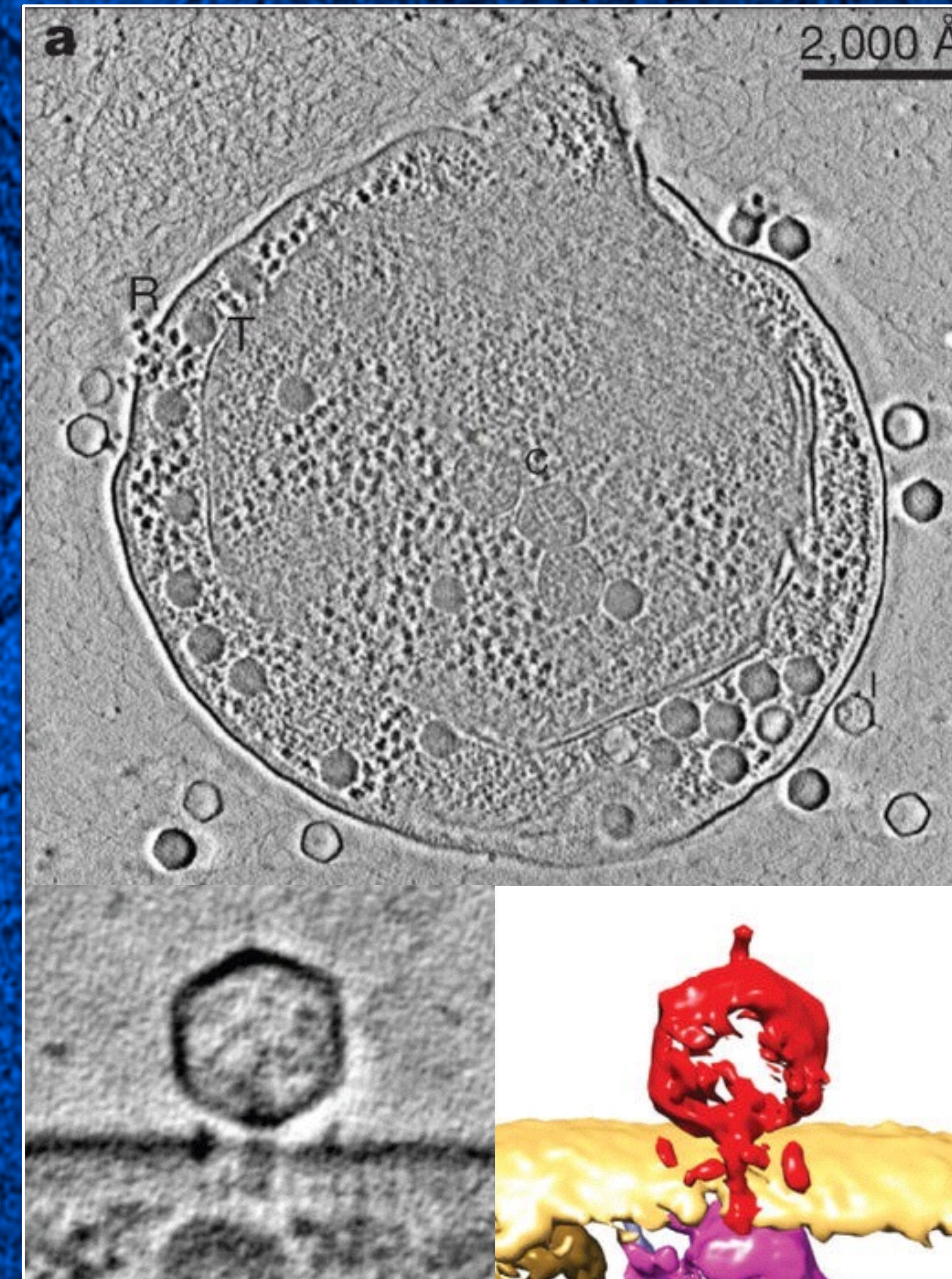




Phage infection remains poorly understood

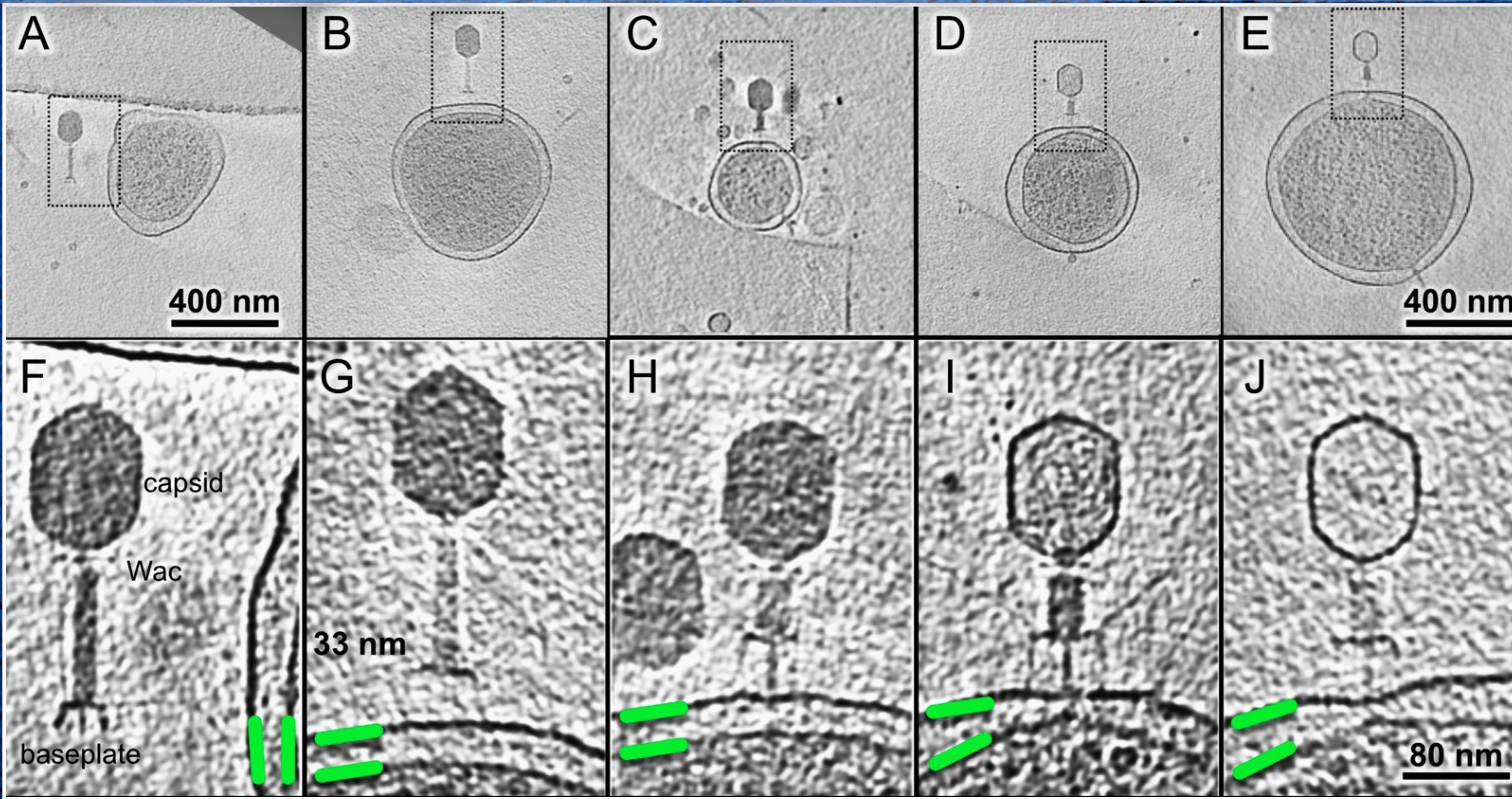


Simon et al. 1967



Dai et al. Nature 2013

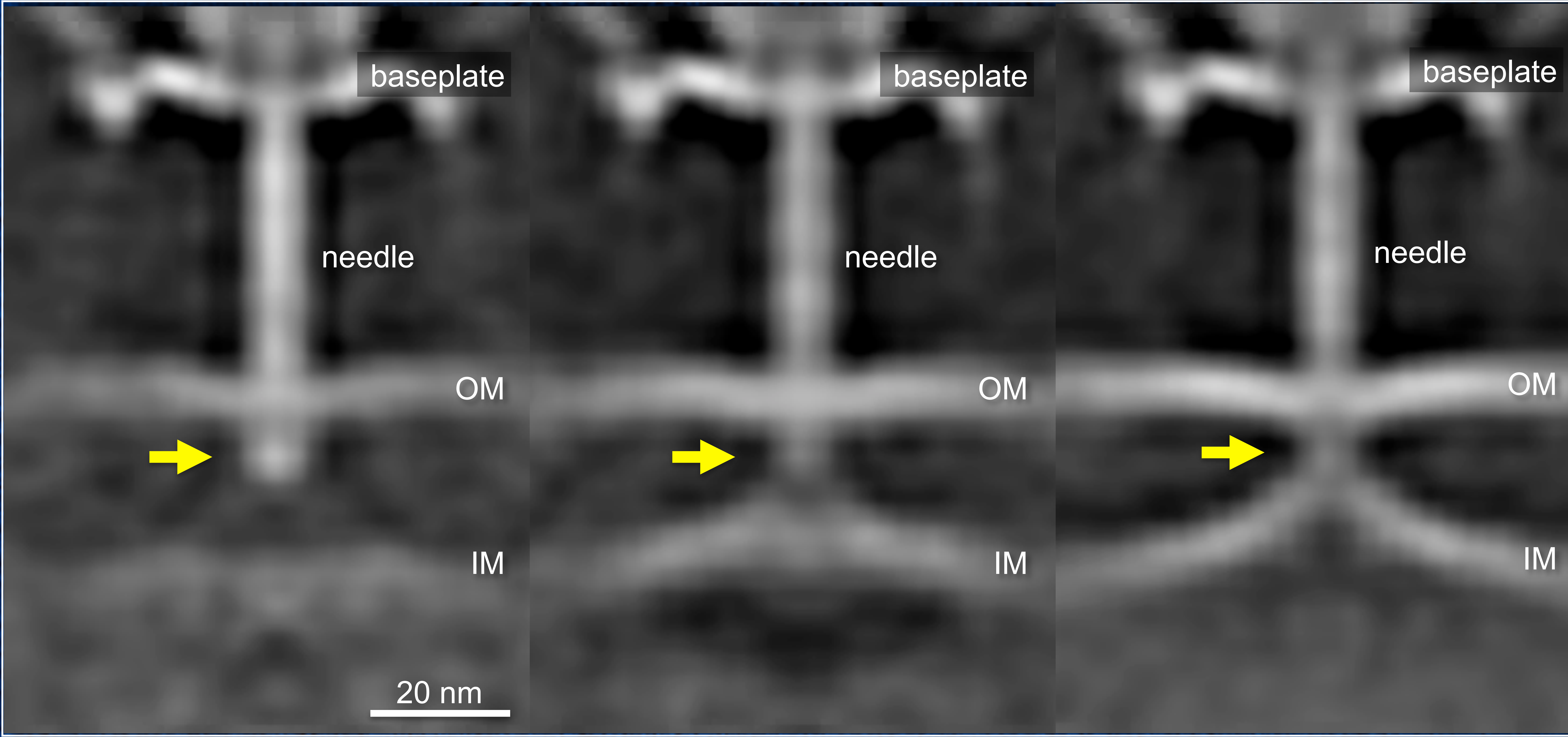
Capturing key intermediates in T4 infection



~30 sec ~1 min ~3 min ~3 min ~10 min

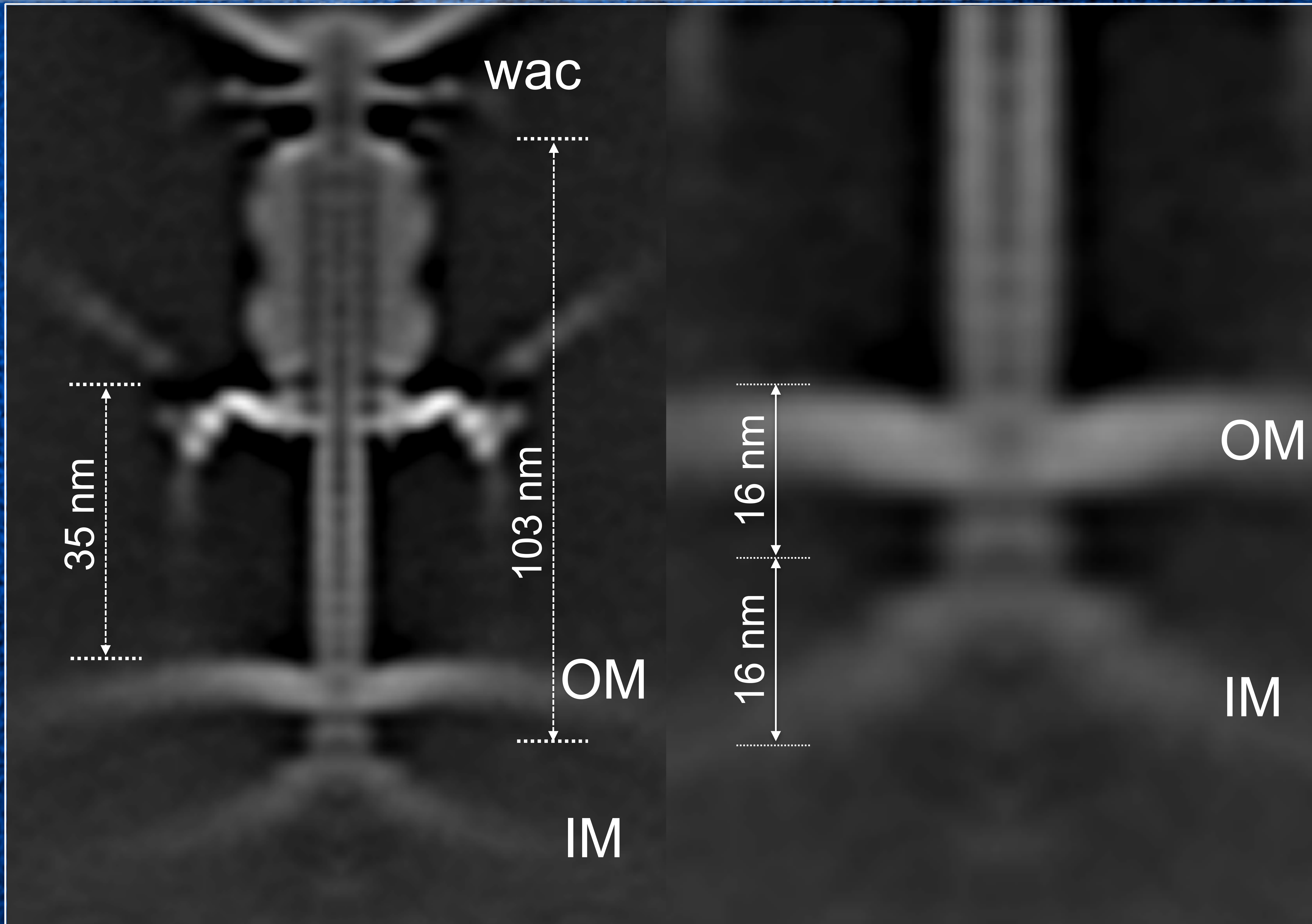
Hu et al. PNAS (2015)

Formation of a trans-envelope channel

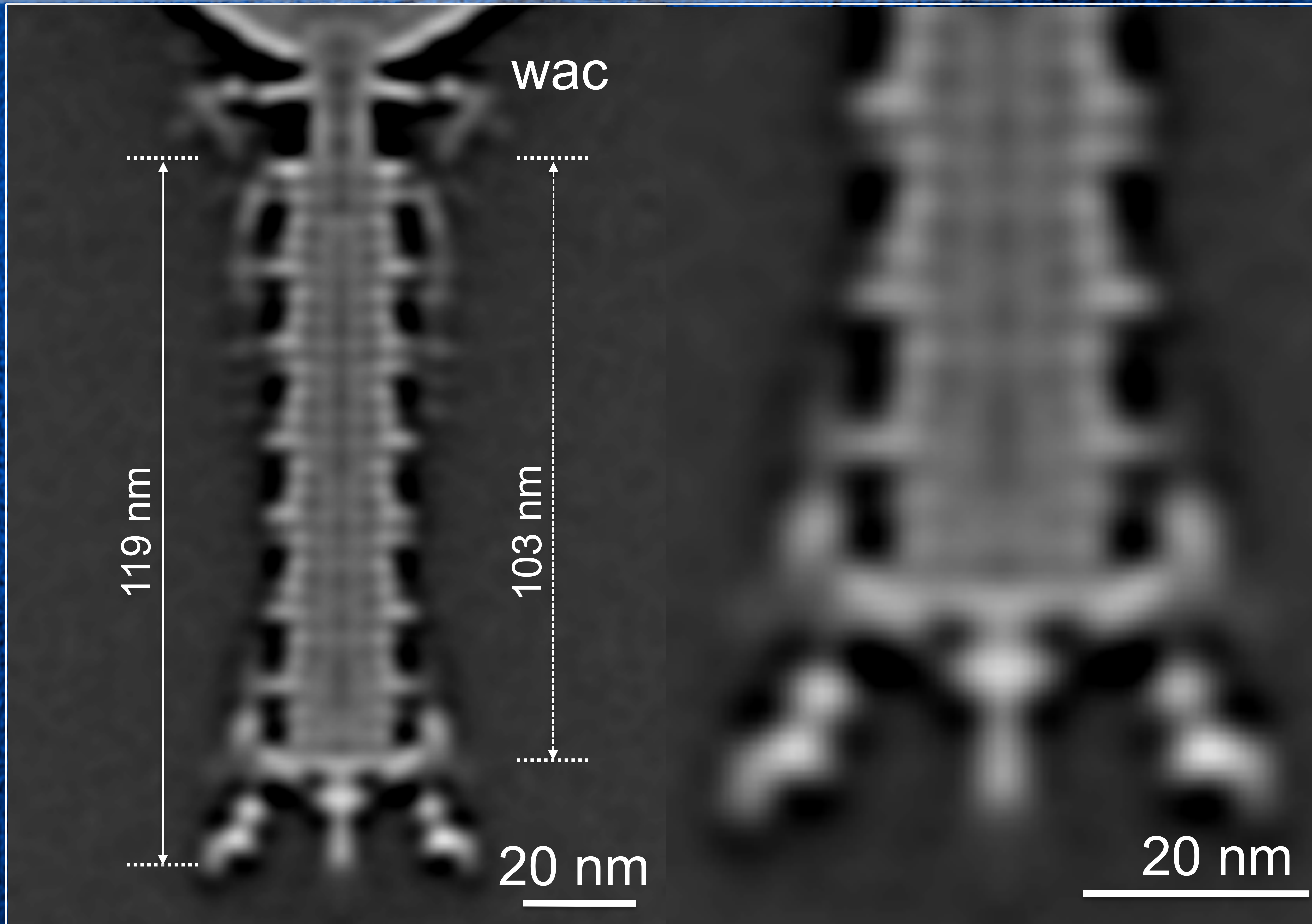


Hu et al. PNAS (2015)

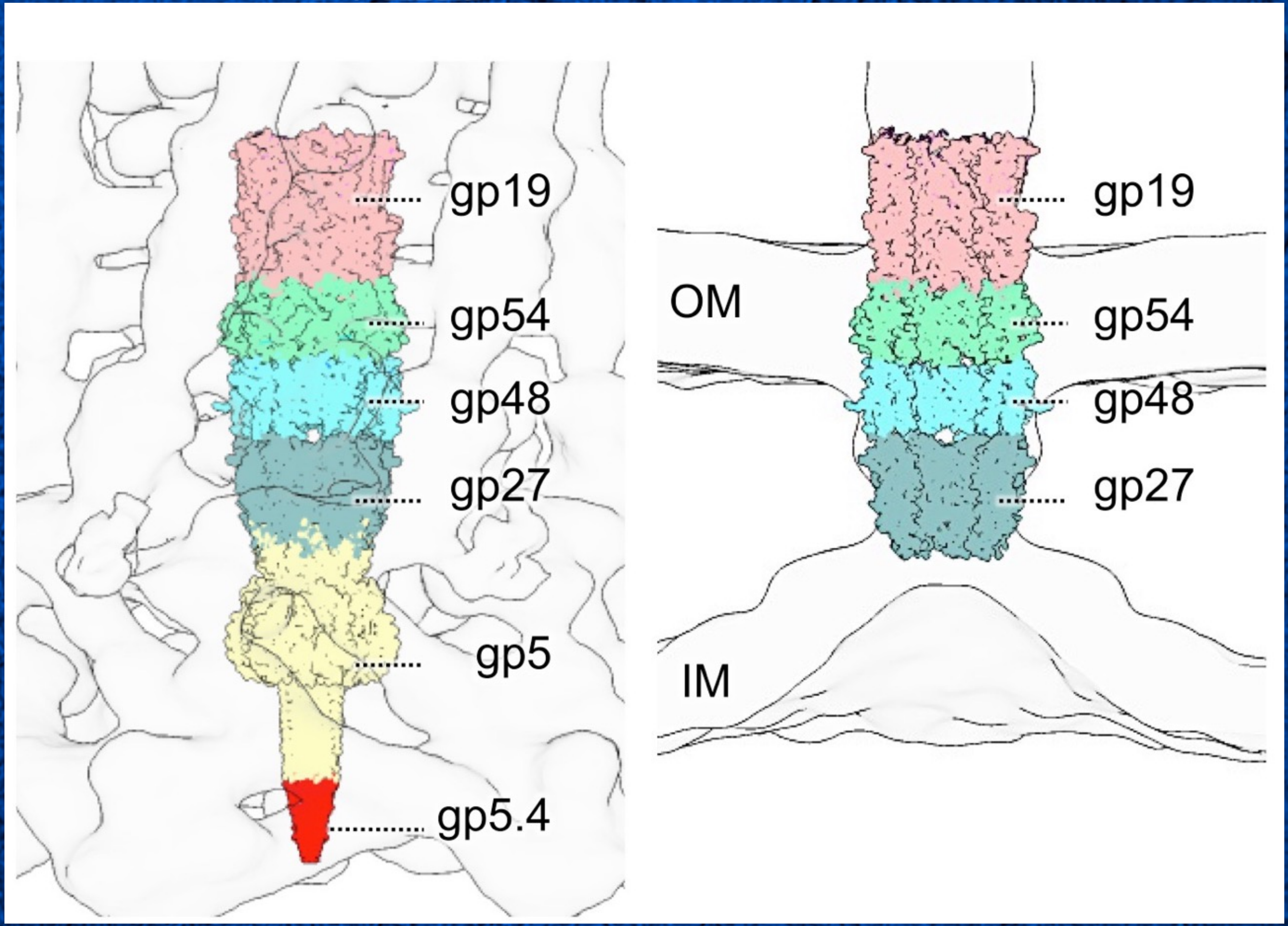
Formation of a trans-envelope channel



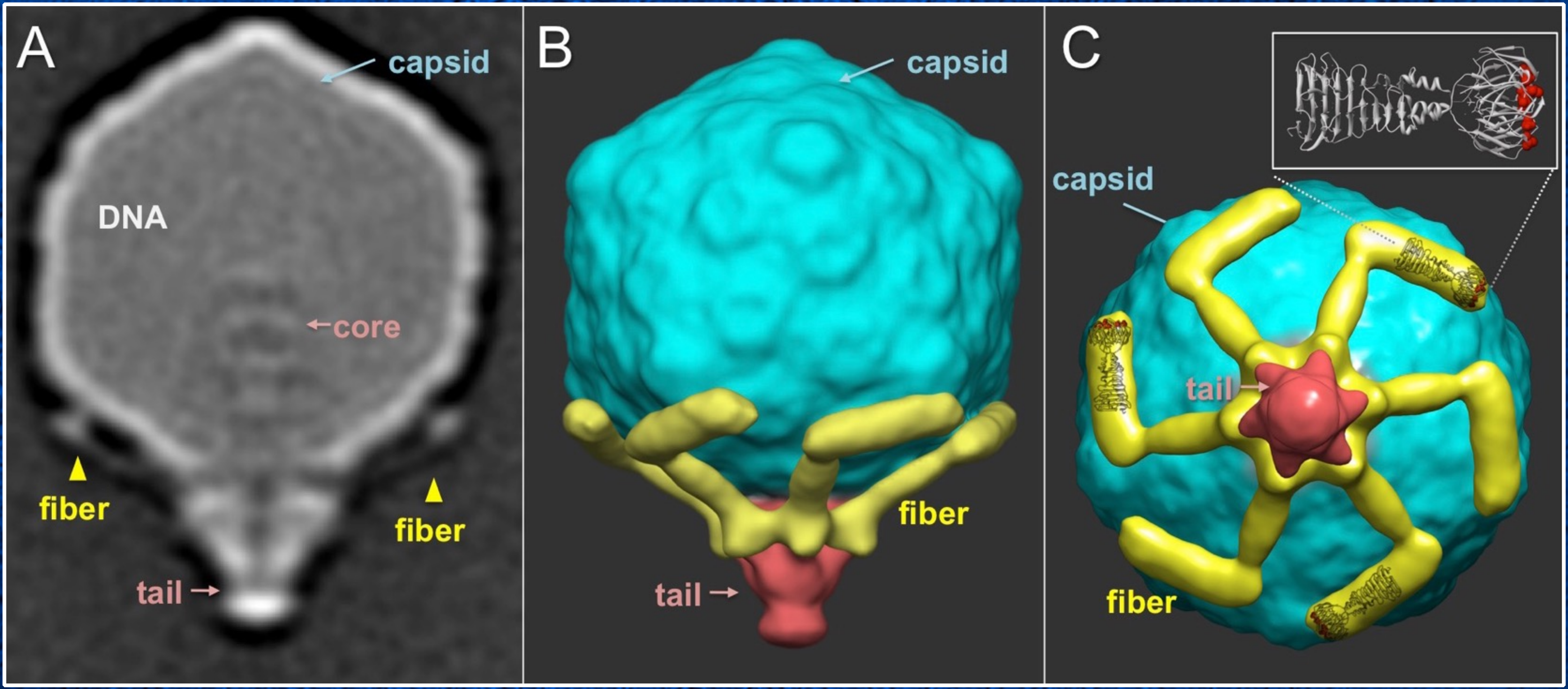
Conformational change during contraction



Model of the trans-envelope channel

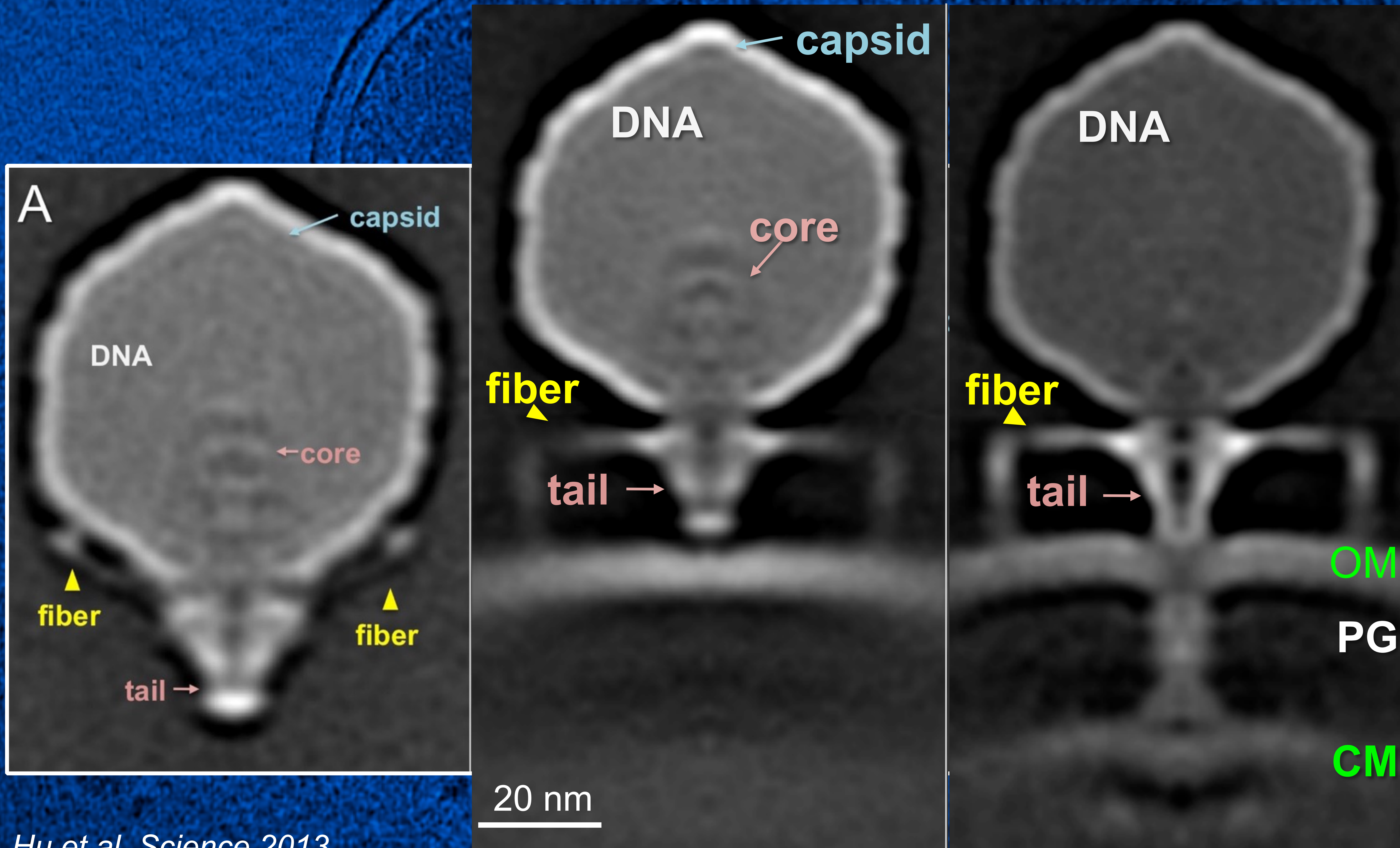


T7 — A short tailed phage

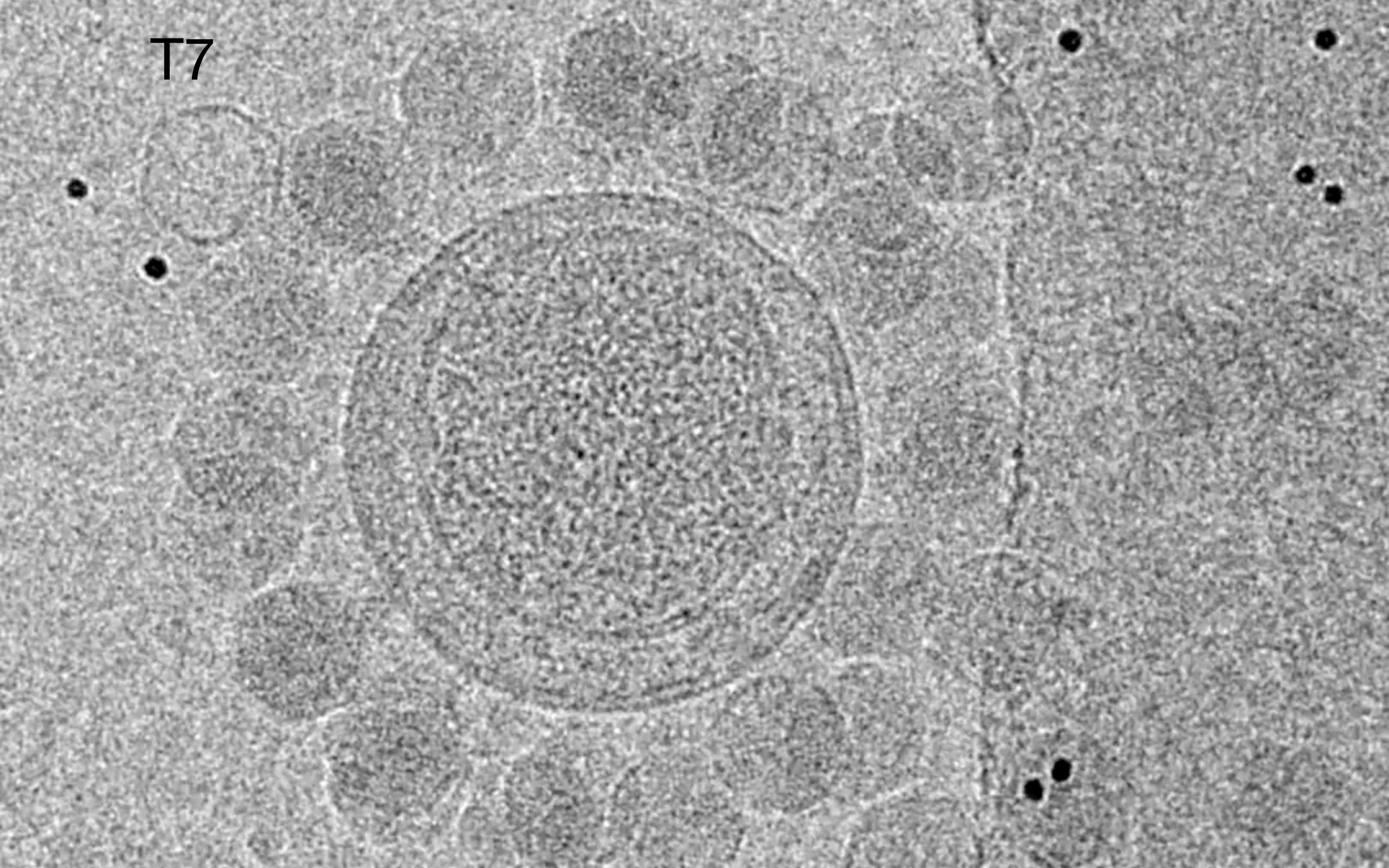


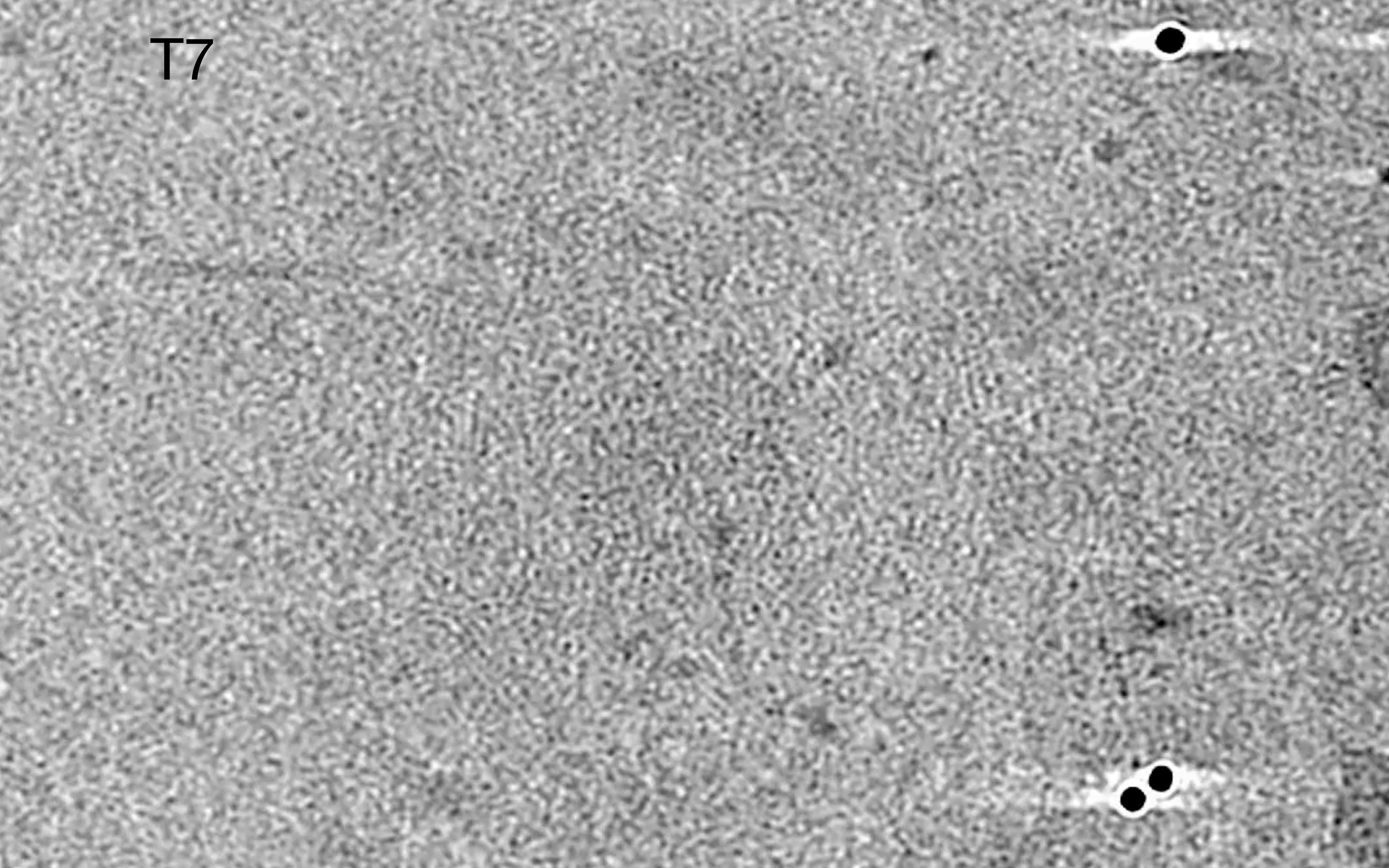
Hu et al. Science 2013

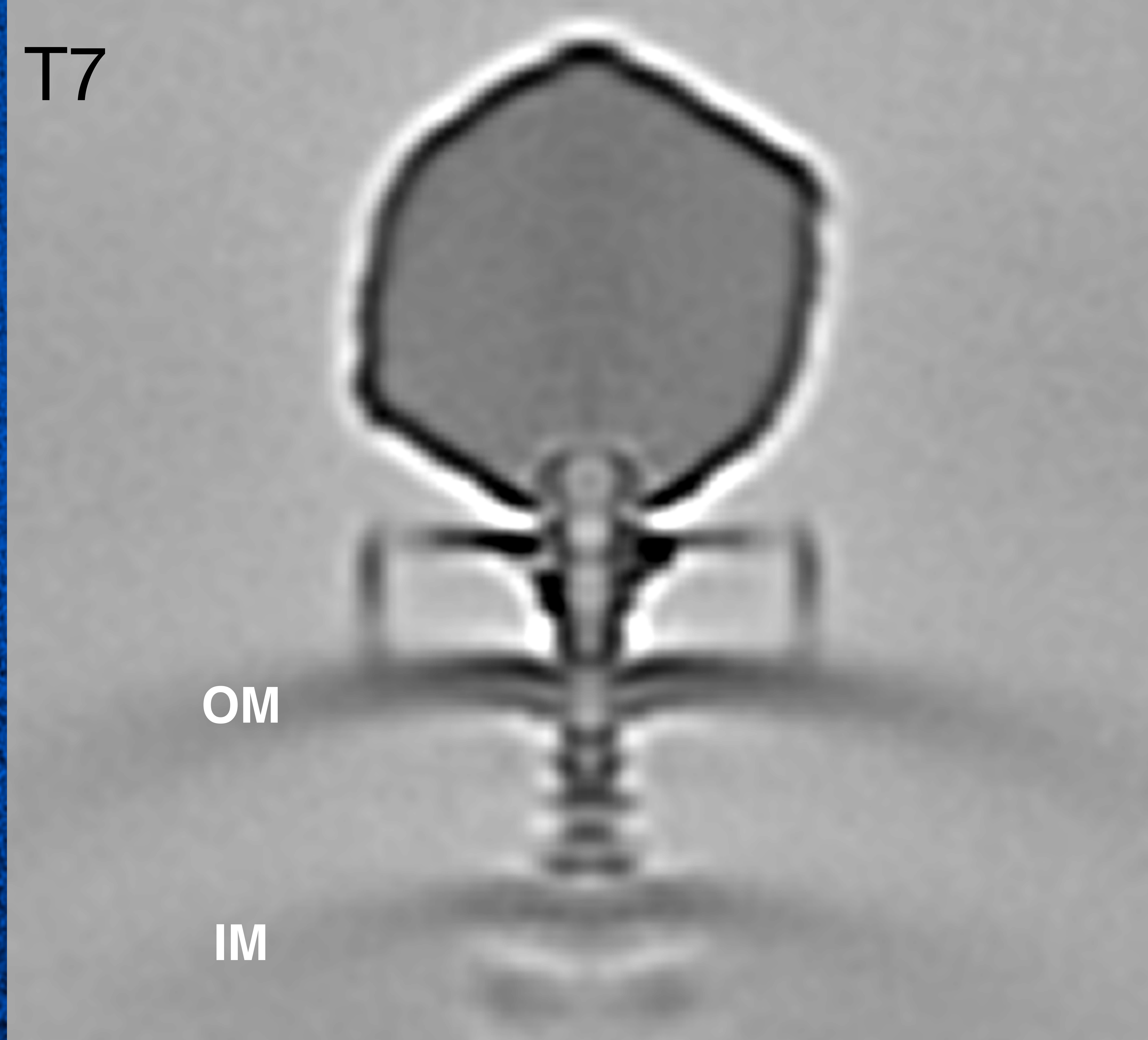
T7 intermediates during infection



Hu et al. Science 2013


T7

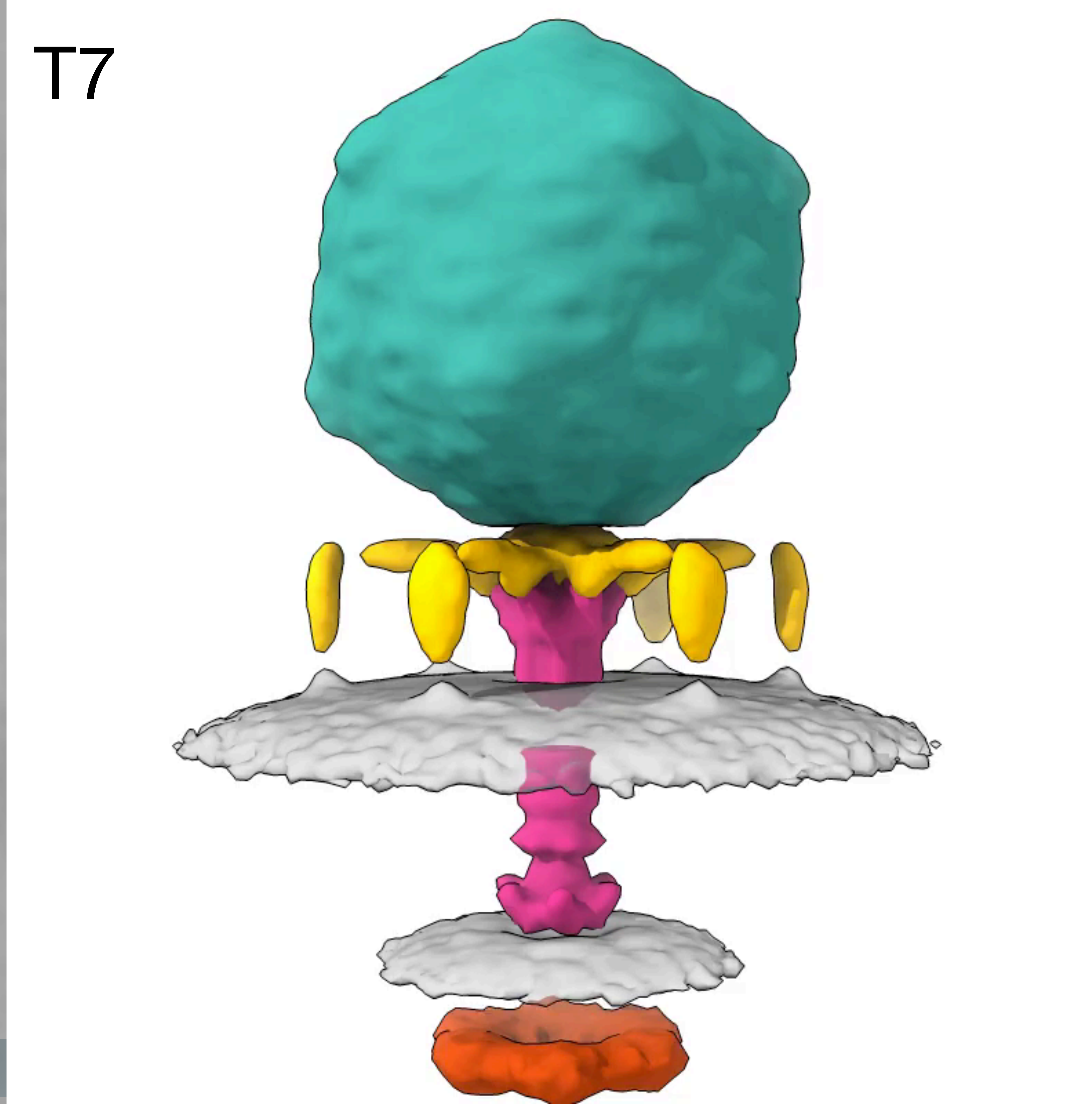

T7



Dustin Morado

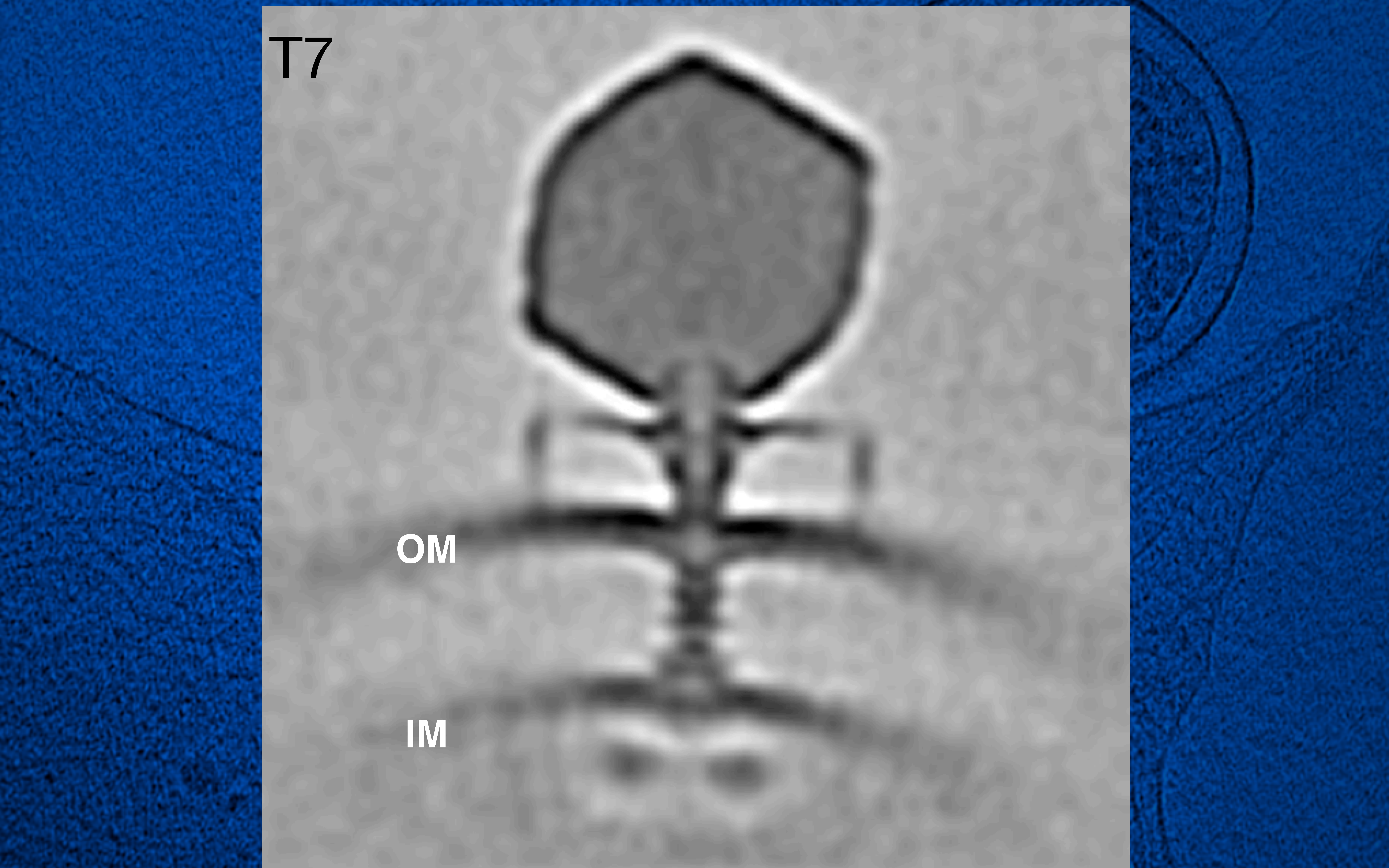
5,706

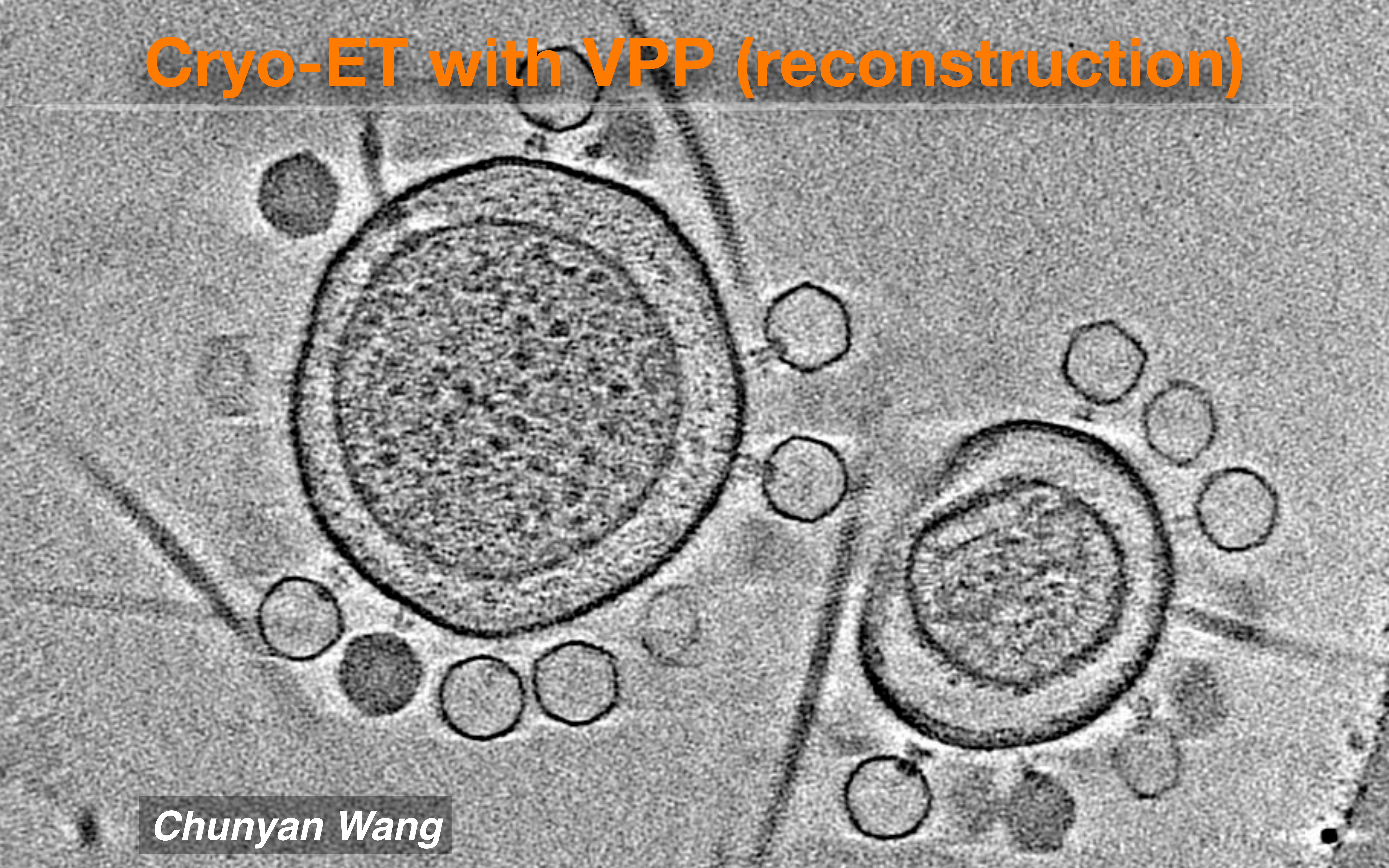
61,000



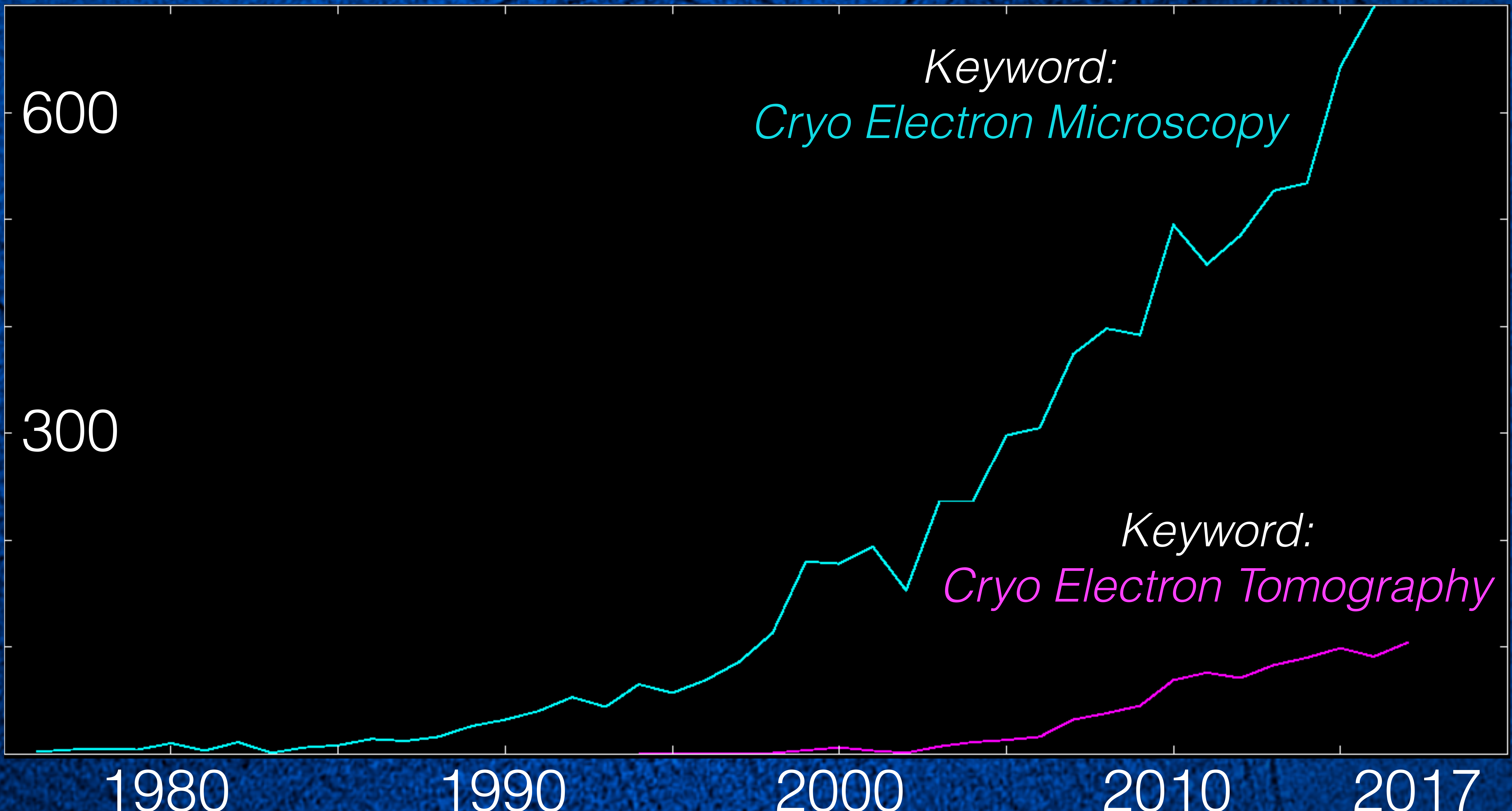
5,706

1,000


T7
OM
IM


Cryo-ET with VPP (reconstruction)
Chunyan Wang

The best is yet to come for cryo-ET



Summary

- ➢ ***We develop high-throughput cryo-ET pipeline to visualize bacteria and their nanomachines in action.***
- ➢ ***Bacterial minicell is a great toolbox for in situ structural determination of nanomachines.***
- ➢ **We determine *in situ* structures of the T3SS machines in *Shigella and Salmonella.***
- ➢ **We reveal novel trans-envelope channels during phage infection.**
- ➢ **Classification is essential for sorting key conformations.**

Acknowledgements (Collaborators)

Shigella:

UNIVERSITY OF KANSAS
William Picking
Wendy Picking

Minicells:

UTHELATH
William Margolin

Salmonella:

YALE UNIVERSITY
Jorge Galan
Maria Lara-Tejero

Phage infection:

UT AUSTIN
Ian Molineux

Borrelia:

UTHEALTH
Steven Norris
Tao Lin
Lihui Gao

SUNY BUFFALO
Chris Li
Kai Zhang

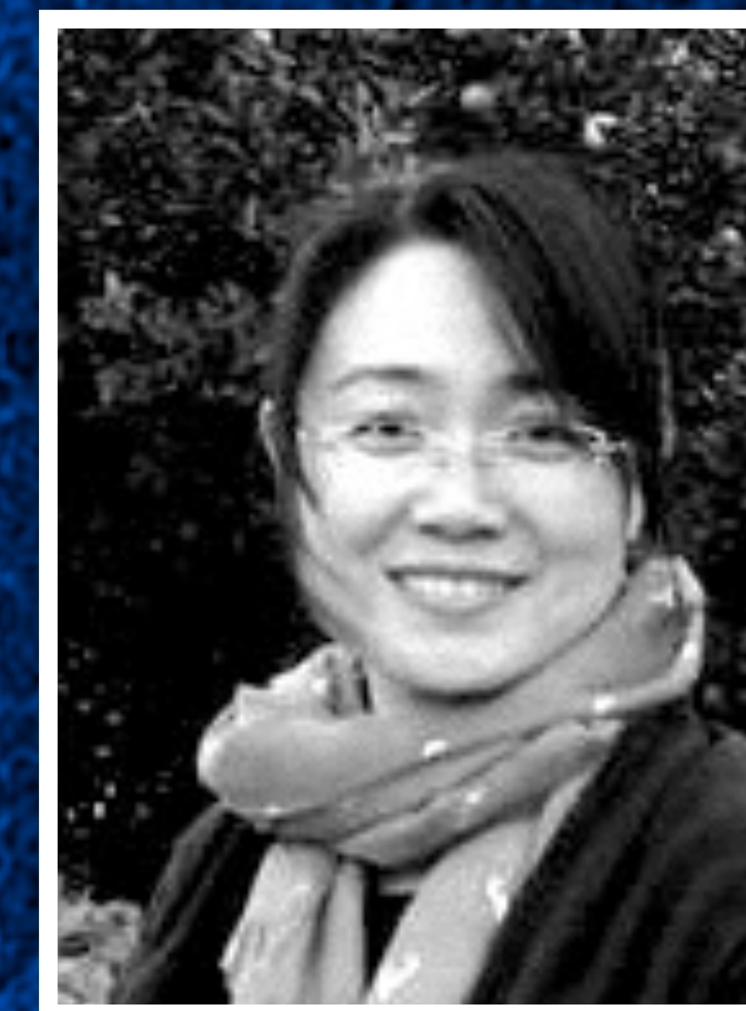
EAST CAROLINA UNIVERSITY
Md Motaleb
Kihwan Moon

WEST VIRGINIA UNIVERSITY
Nyles Charon

Acknowledgements

Zhuan

Chunyan



Xia (Cathy)

Raphael

Bo Hu

Xiaowei Zhao

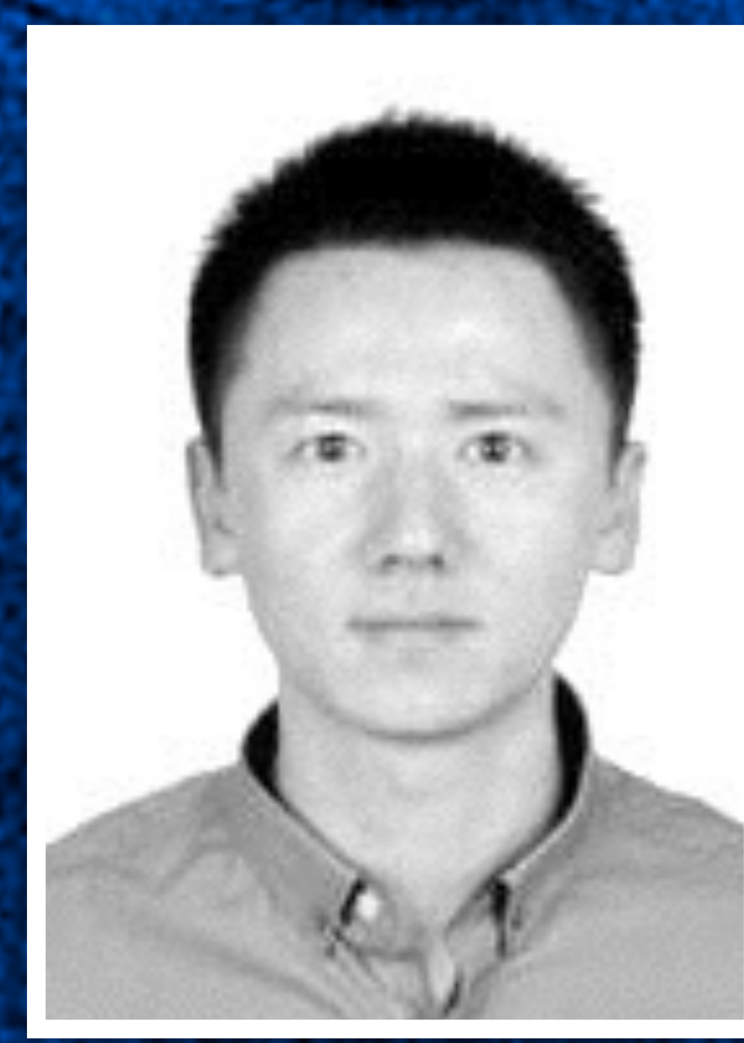
Dustin Morado

Madeline Farley

Jiagang Tu

Wendy Wang

Shiwei



Yunjie

Wenwei

Thanks Very Much !

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Basic Discoveries for Better Health

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