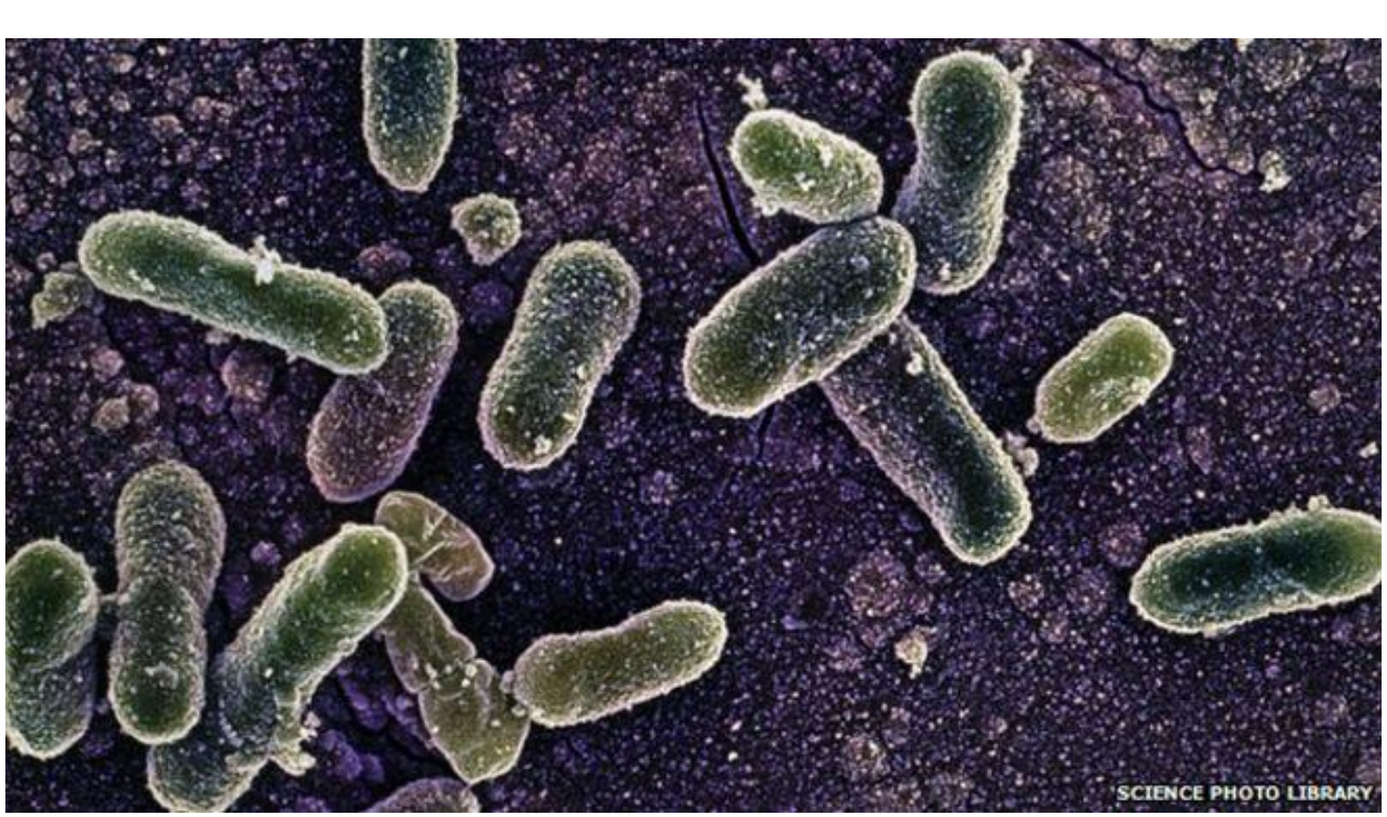
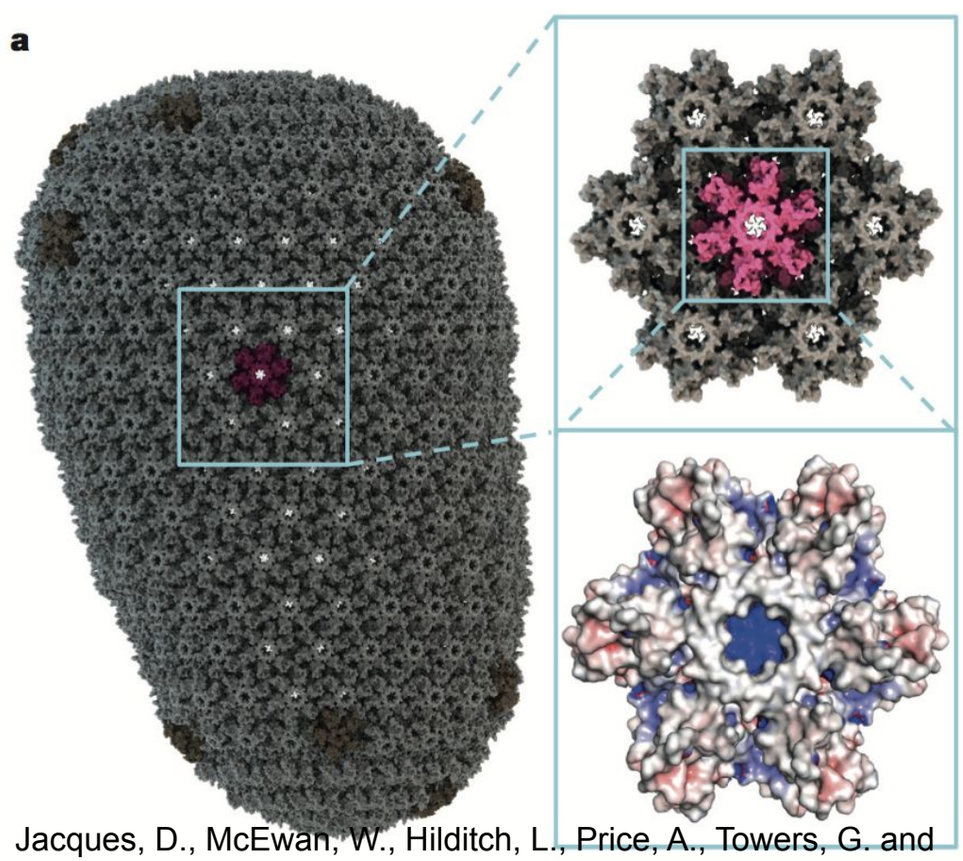
Pathogenesis of bacteria and viruses



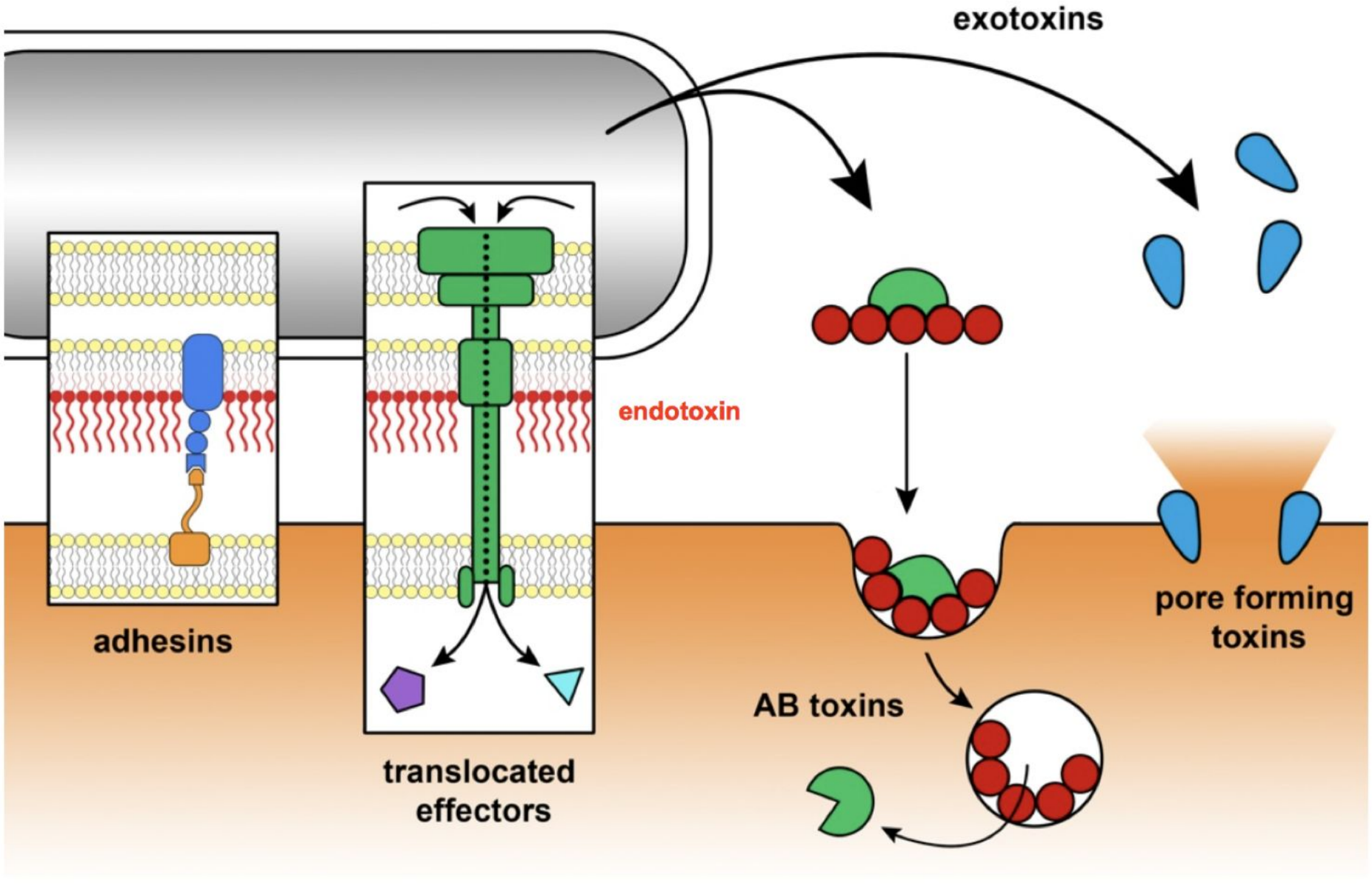
http://www.bbc.com/news/health-28804267



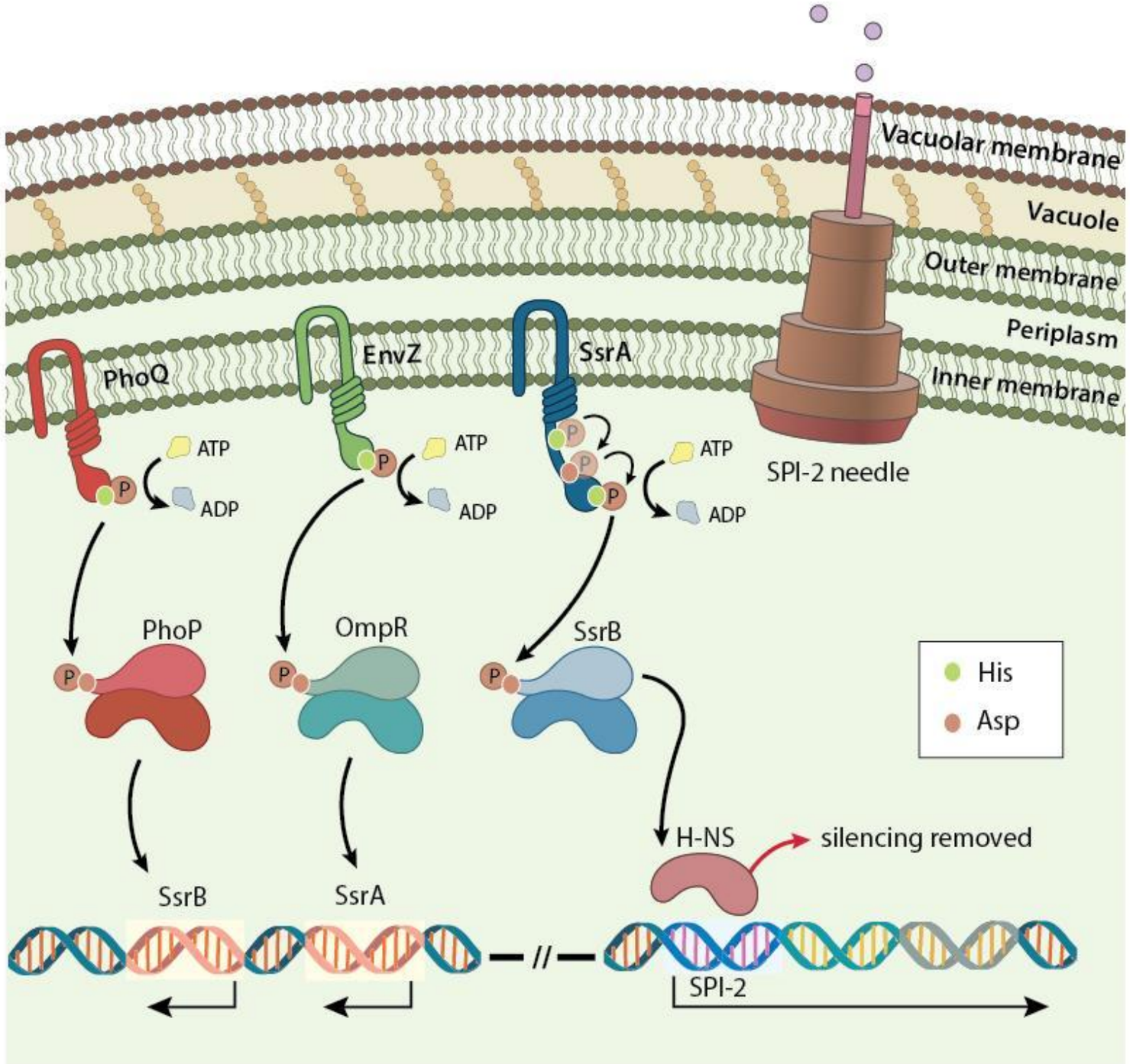
Jacques, D., McEwan, W., Hilditch, L., Price, A., Towers, G. and James, L. (2016). HIV-1 uses dynamic capsid pores to import nucleotides and fuel encapsidated DNA synthesis. *Nature*,

Bacteria

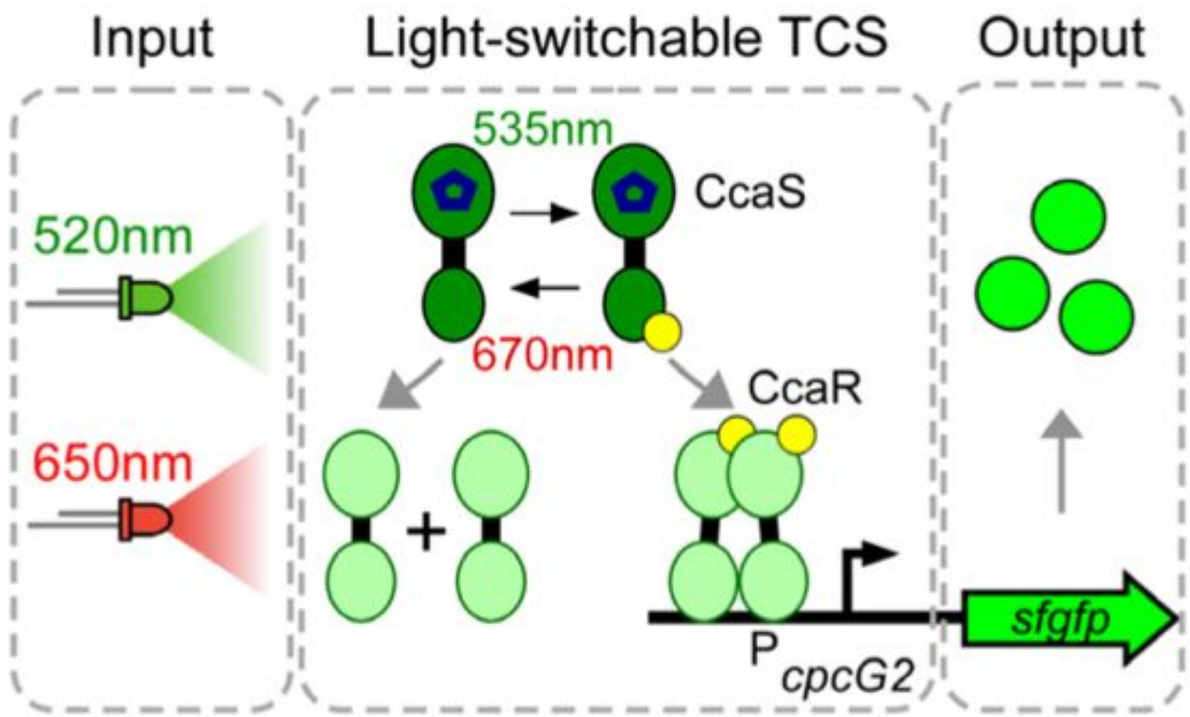
Virulence factors



Regulation of virulence factors: two-component systems (TSS)



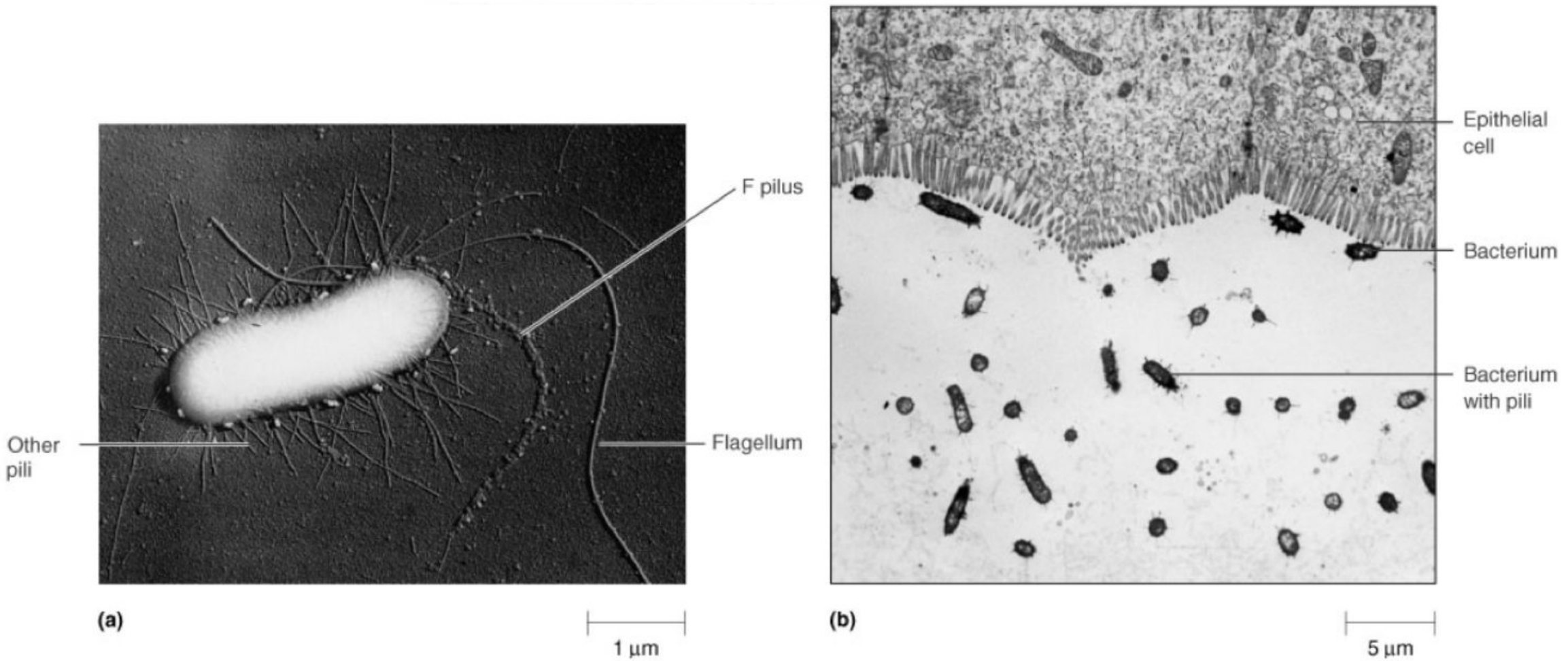
A bit of synthetic biology…



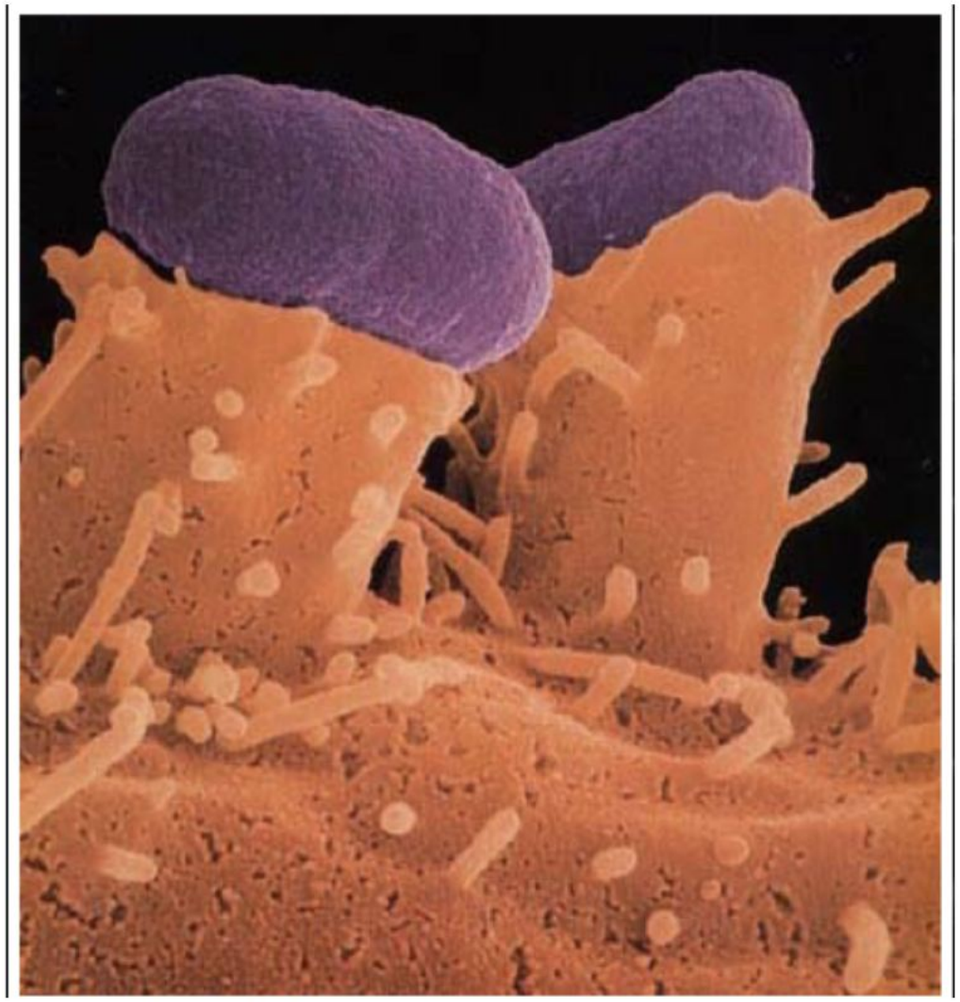
Schmidl, S., Sheth, R., Wu, A. and Tabor, J. (2014). Refactoring and Optimization of Light-SwitchableEscherichia coliTwo-Component Systems. *ACS Synthetic Biology*, 3(11), pp.820-831.

Key events of pathogen-host interaction

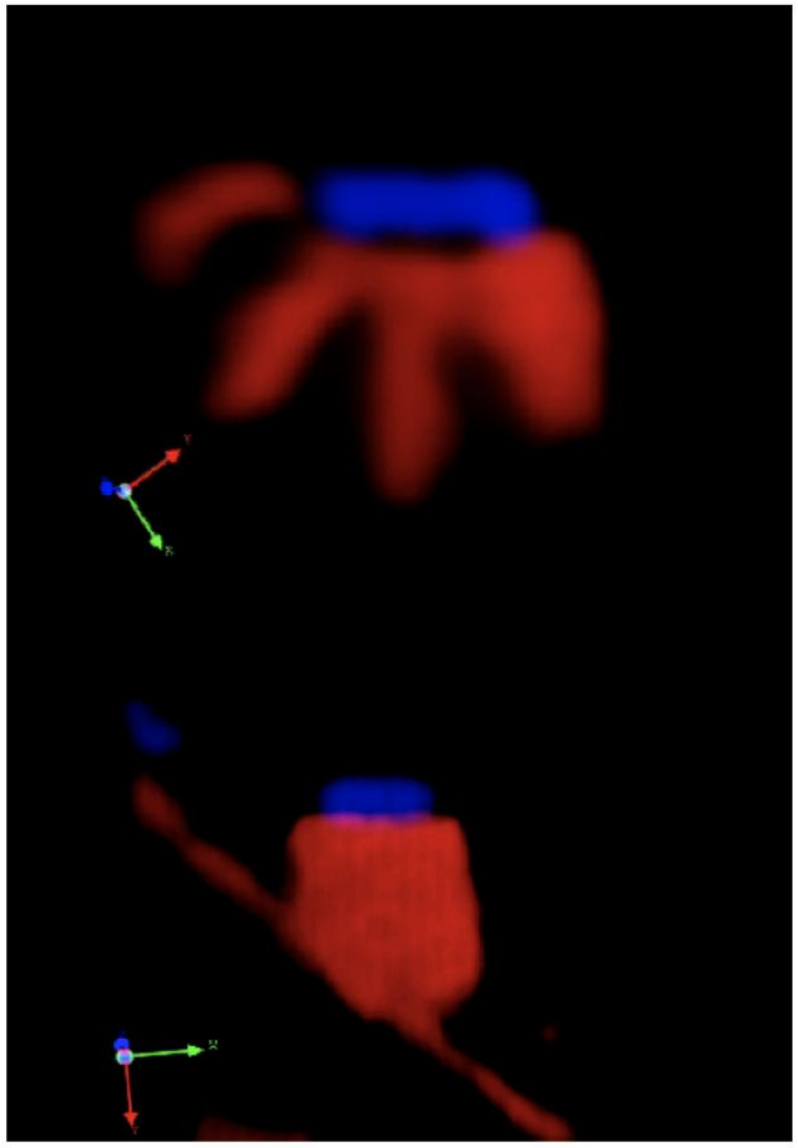
1. Colonisation - invasion
2. Multiplication
3. Transmission
4. Damage



Uropathogenic *E. coli* binding a kidney receptor with adhesins at the top of P-pili



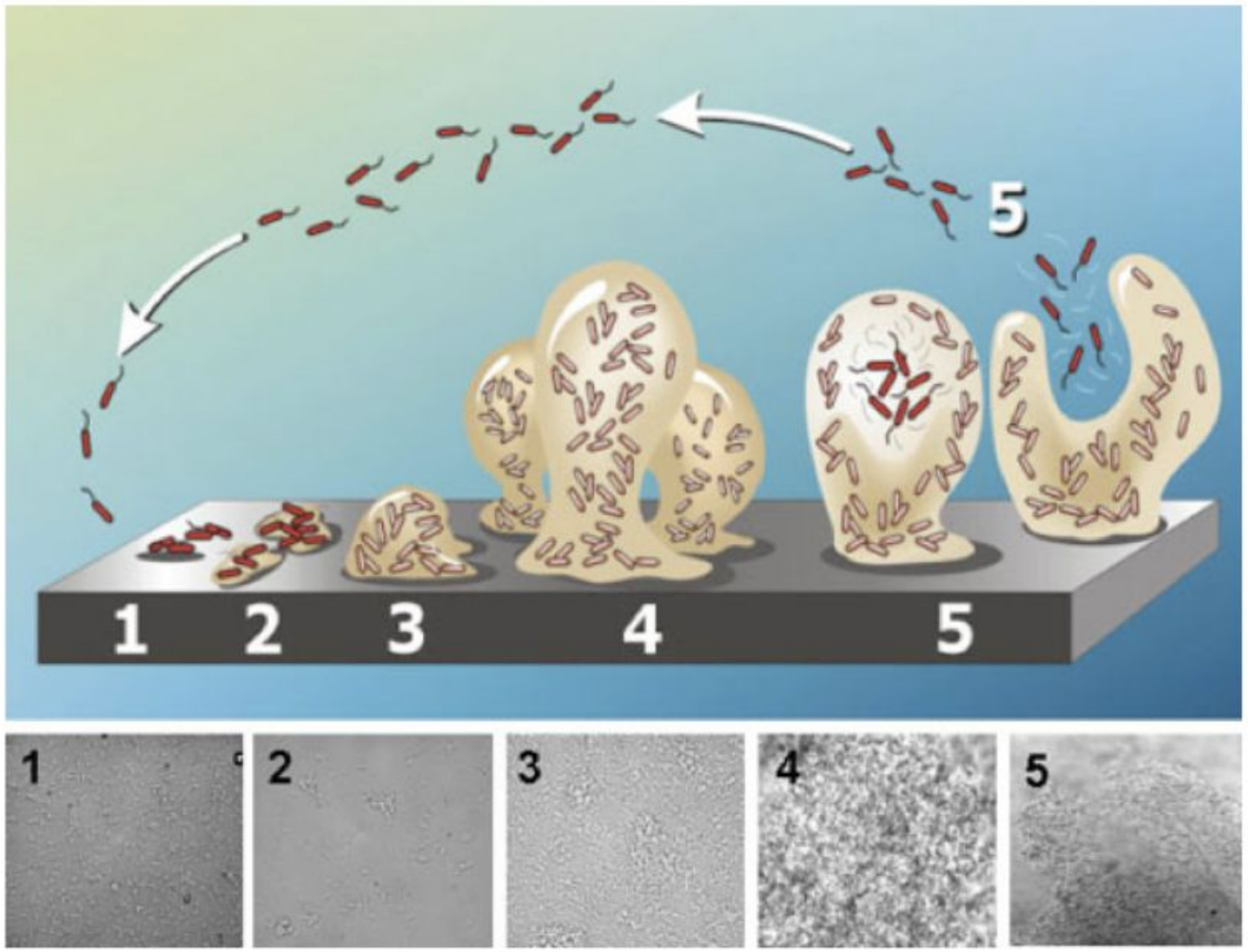
scanning electron microscopy



cytoskeleton
enteropathogenic *E.coli*

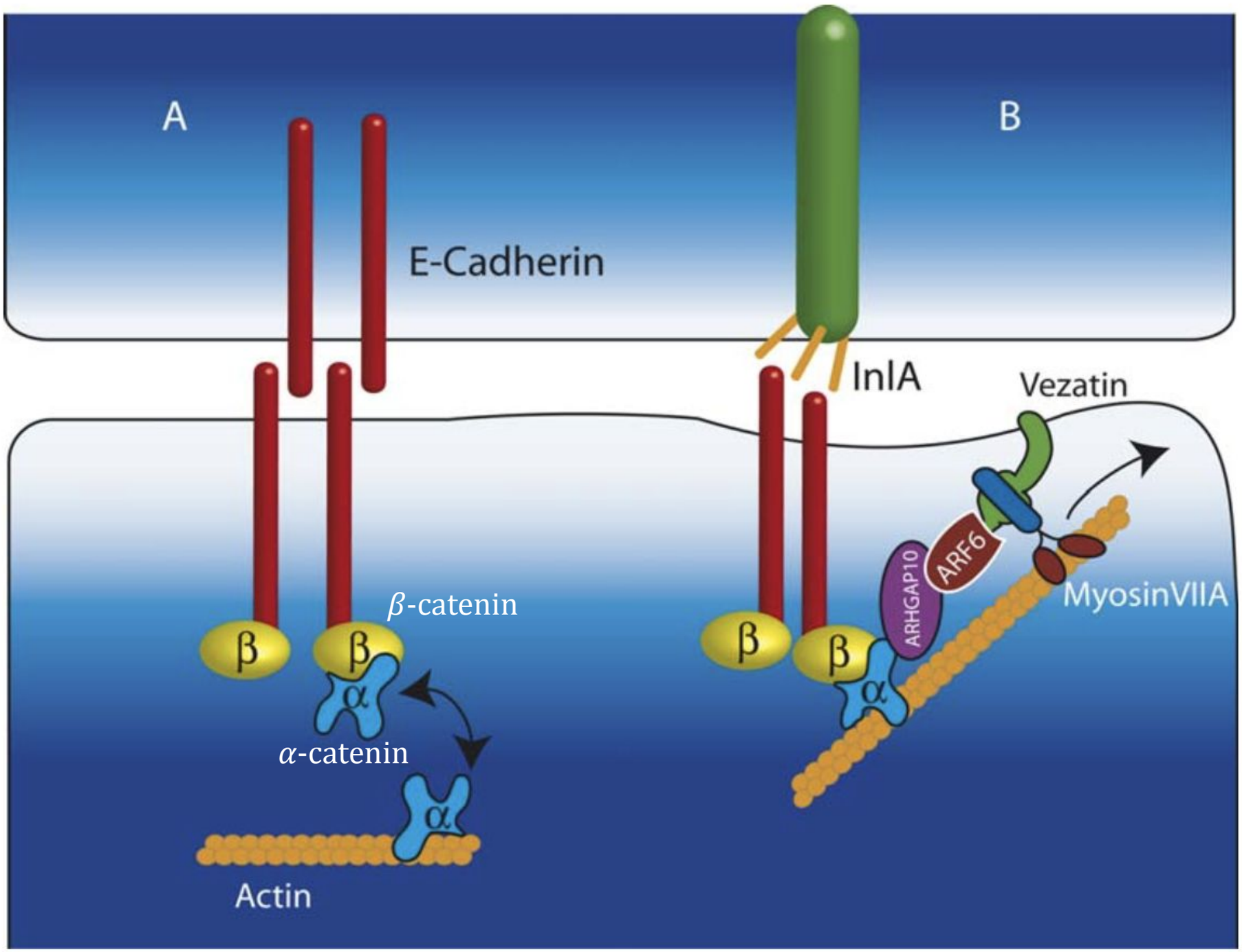
Enteropathogenic *E. coli* build specialised structures for adhesion

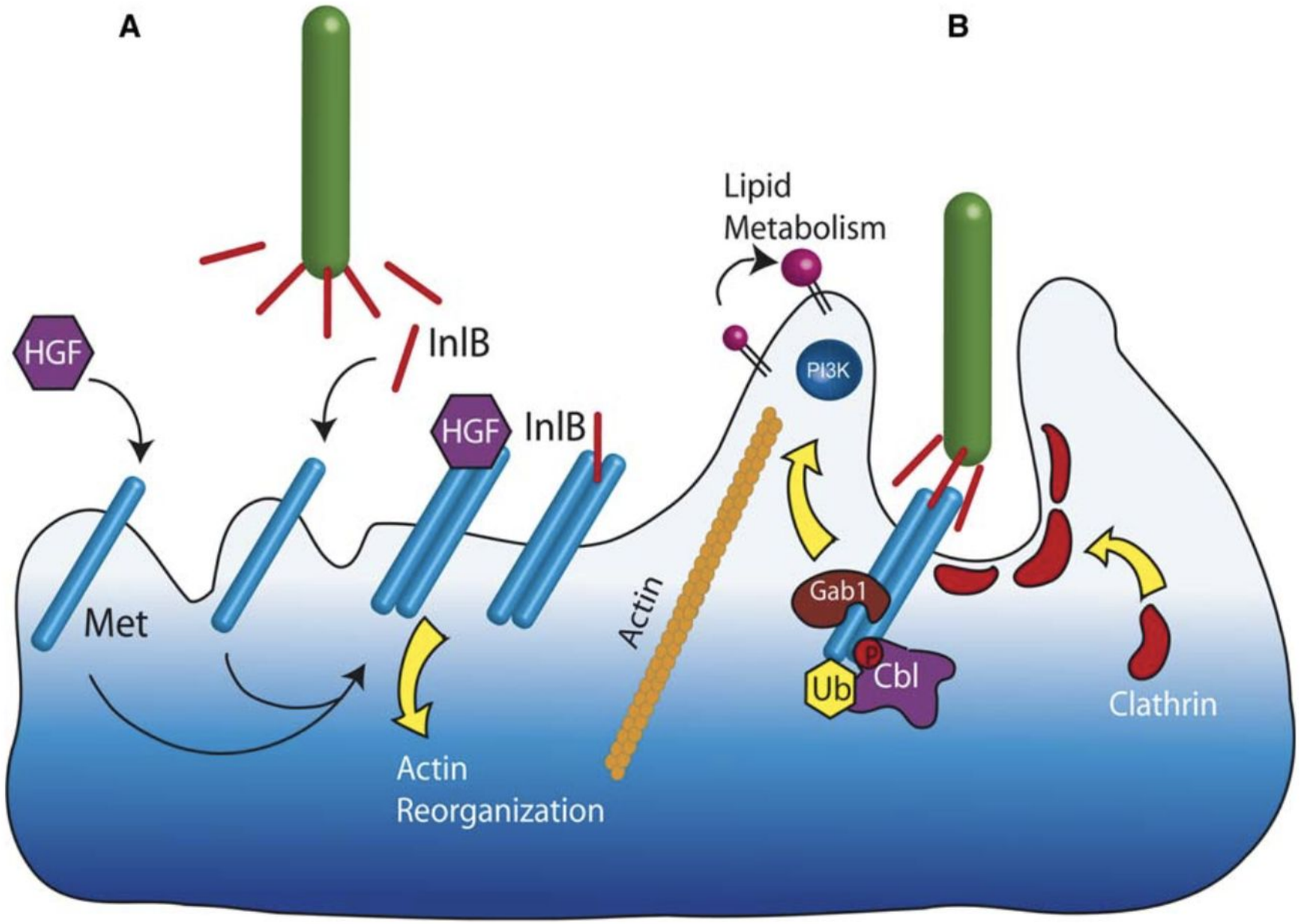
Biofilms



Invasion

"Zipper mechanism". ***Listeria*** invading non-phagocytic cells.

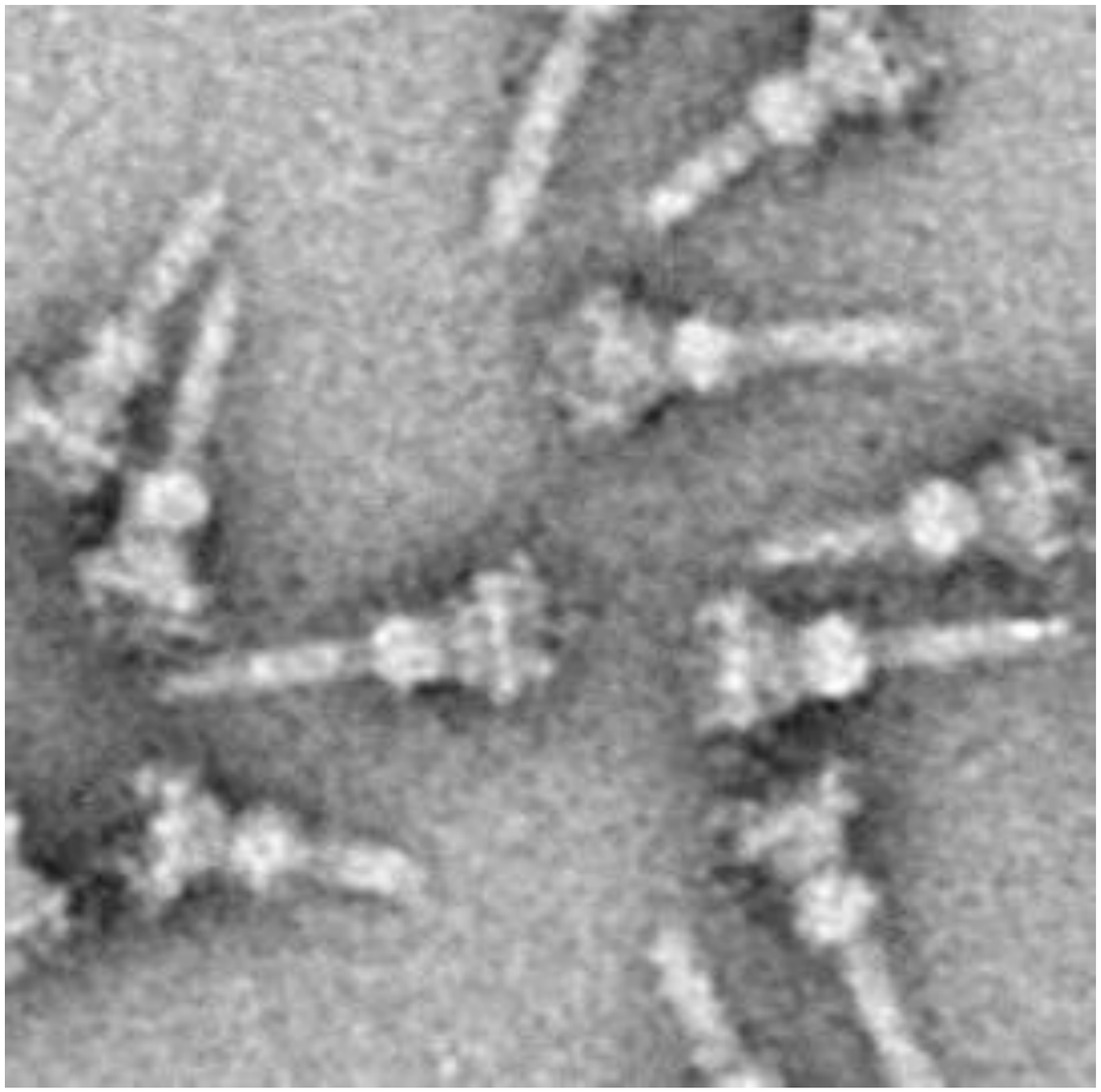




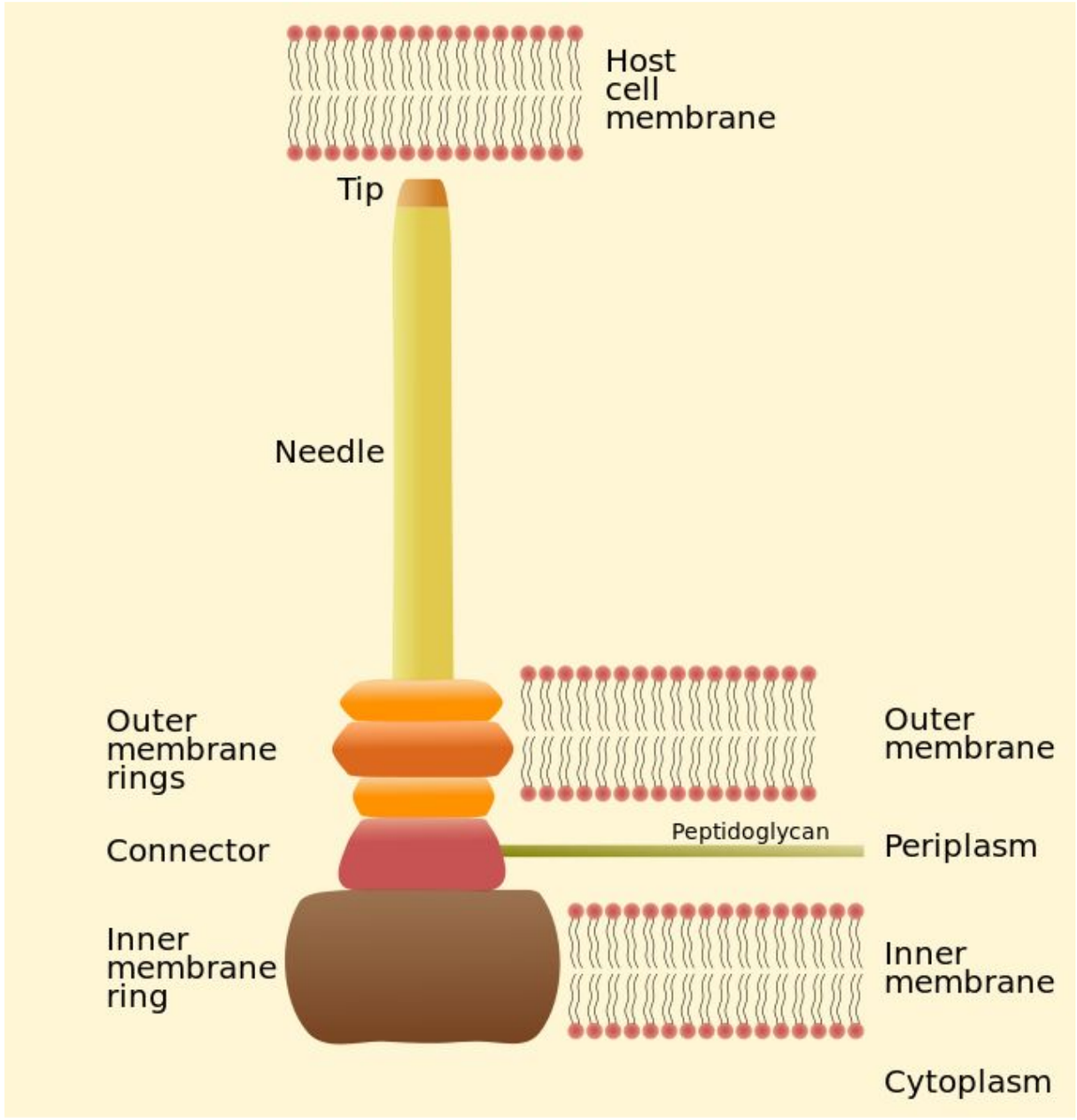
Bonazzi, M. and Cossart, P. (2006). Bacterial entry into cells: A role for the endocytic machinery. *FEBS Letters*, 580(12), pp.2962-2967.

“Trigger mechanism”. *Salmonella*.

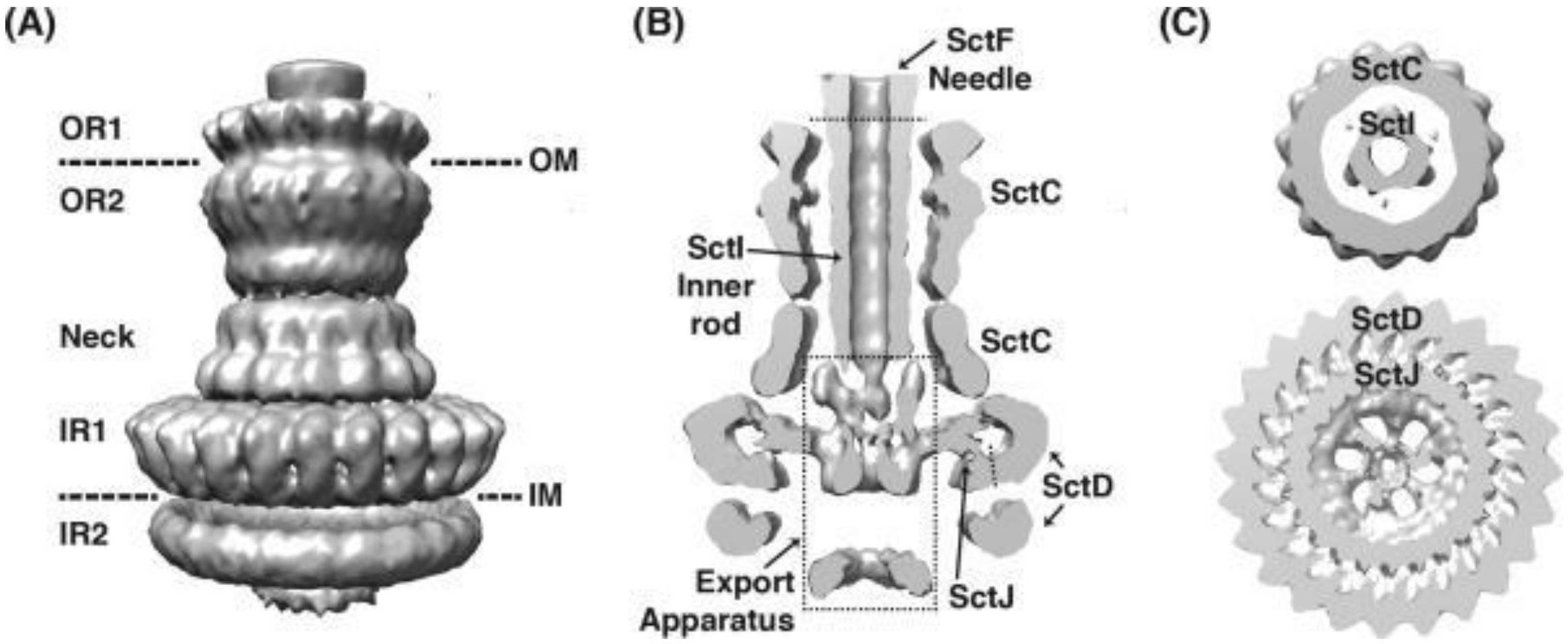
Type III Secretion System



Transmission EM micrograph of *Salmonella* possessing T3SS



Cryo-EM micrograph of T3SS



Abbreviations used: OR, outer ring; IR, inner ring; OM, outer membrane; IM, inner membrane. (B) An axial section through the map in (A). (C) Transverse sections through the map in (A) at the level of the neck (top) and IR1 (bottom).

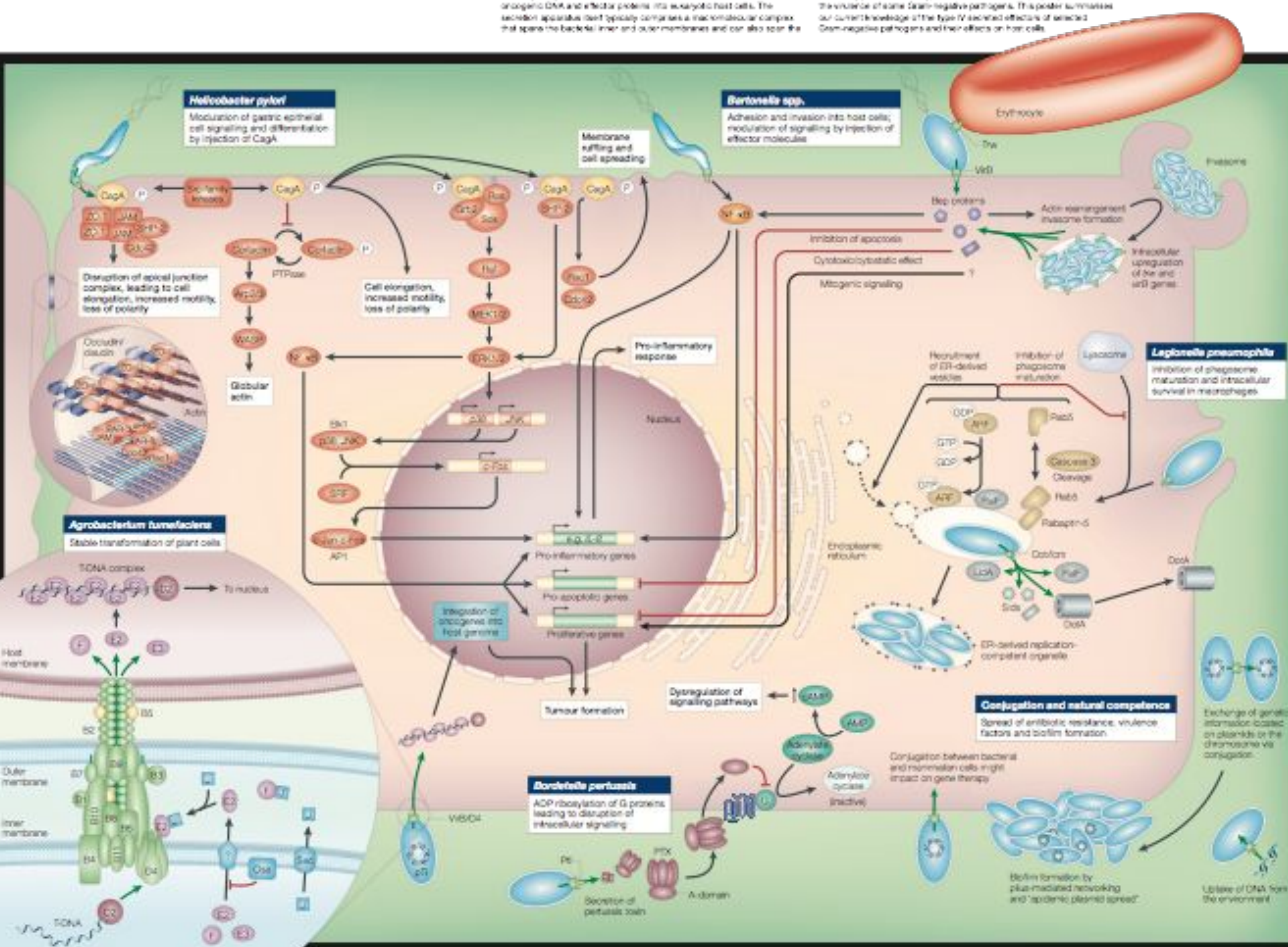
Notti, R. and Stebbins, C. (n.d.). The Structure and Function of Type III Secretion Systems. *Virulence Mechanisms of Bacterial Pathogens, Fifth Edition*, pp.241-264.

nature REVIEWS MICROBIOLOGY

Type IV secretion

Anja Seubert, Rino Rappuoli and Antonello Covacci

The type IV secretion systems of Gram-negative bacteria are evolutionarily related to bacterial conjugation systems. Gram-negatives use type IV secretion systems for a variety of biological functions including the exchange of genetic material with other bacteria and the translocation of oncogenic DNA and effector proteins into eukaryotic host cells. The secretion apparatus itself typically comprises a macromolecular complex that spans the bacterial inner and outer membranes and can also span the membrane of eukaryotic host cells. This assembly is typically composed of up to 12 proteins, and recent research has revealed detailed information on the structure and assembly of the secretion apparatus. It is becoming increasingly clear that the type IV secreted effectors play important roles in the virulence of some Gram-negative pathogens. This poster summarizes our current knowledge of the type IV secreted effectors of selected Gram-negative pathogens and their effects on host cells.



Helicobacter pylori

The gastric pathogen *H. pylori* uses its type IV secretion system (T4SS) (the so-called cag system) in the colonization of gastric epithelial cells. The secreted effector CagA has a dramatic effect on host cells, including major changes in cellular morphology, and contributes to chronic gastric inflammation and possibly also the formation of gastric carcinoma.

Bartonella

Bartonella spp. require two different T4SSs for pathogenicity. The Trw system, where extensive gene duplication creates variant pilus subunits, is necessary for colonization of erythrocytes. The VirB system and its secreted *Bartonella* effector proteins (Beps) are believed to be responsible for most of the cellular effects of the interaction of *Bartonella* with host endothelial cells: actin rearrangements, resulting in invasome formation, activation of a pro-inflammatory response and inhibition of apoptosis. Together with a T4SS-independent mitogenic stimulus, these effects result in endothelial cell survival and proliferation, and the formation of vasoproliferative tumours.

Legionella pneumophila

L. pneumophila can replicate within macrophages by interfering with the normal pathway of endocytosis. The functions of two effectors translocated by the Dot/Icm T4SS system have been identified. RalF is a guanine nucleotide exchange factor that recruits ADP ribosylation factor (ARF) proteins to the *Legionella*-containing vacuole. LidA is believed to be involved in maintaining the integrity of the *Legionella* cell. A pool of other translocated effectors (Sids; substrate of Icm/Dot transporter) were identified and their functions remain to be explored. It has been shown recently that activation of caspase 3 is dependent on the Dot/Icm T4SS. Caspase 3 cleaves Rabaptin 5, thereby preventing Rab5 recruitment to the phagosomal membrane and inhibiting endocytic fusion.

Agrobacterium tumefaciens

The VirB/D4 system in the plant pathogen *A. tumefaciens* is the prototypical T4SS. *A. tumefaciens* translocates oncogenic single-stranded DNA (T-DNA) into a variety of dicotyledonous plants, causing tumours known as crown galls. The T-DNA is translocated as a nucleoprotein complex with VirD2, which, along with VirE2, ensures that the T-DNA is translocated to the nucleus. Other secreted effectors include the F-box protein VirF, the intracellular target of which remains unknown, and VirE3, which is also of unknown function.

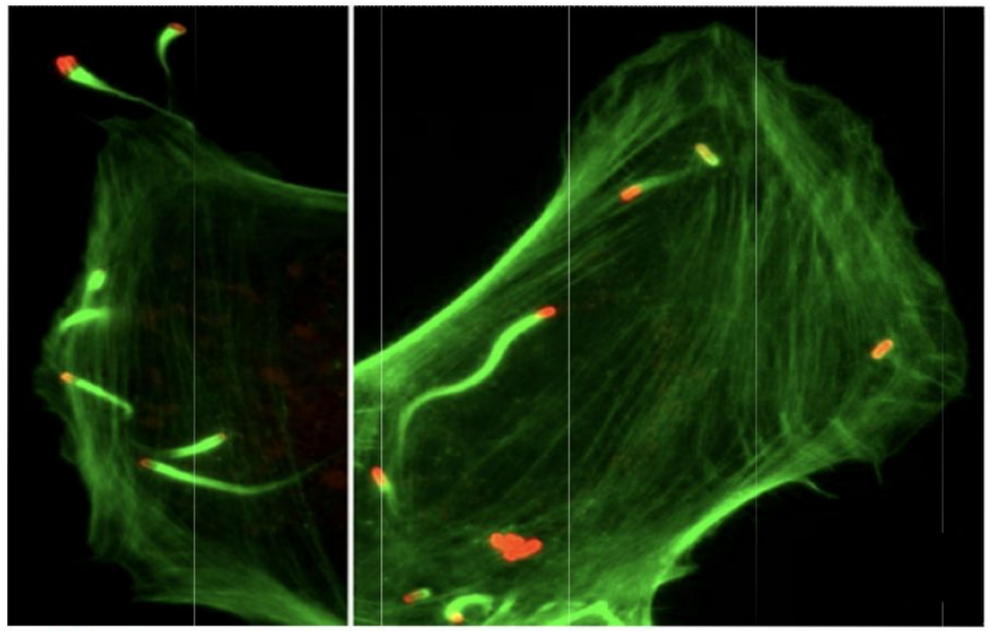
Bordetella pertussis

The Ptl T4SS found in *B. pertussis*, the causative agent of whooping cough, differs from the other systems shown in this poster as it secretes its effector protein — the pertussis toxin (PTX) — into the extracellular milieu. The downstream effects of PTX include ADP ribosylation of the inhibitory G protein (Gi), which increases the intracellular levels of cAMP and modulates intracellular signalling pathways, leading to cell death.

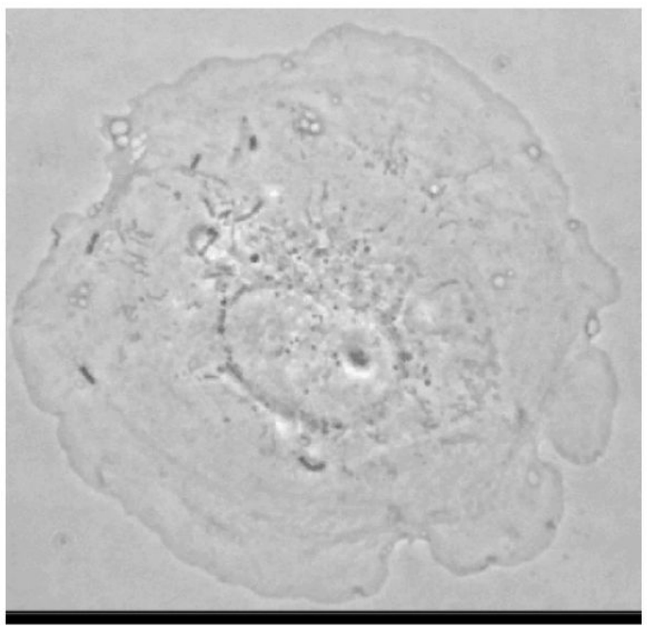
FURTHER READING
[illegible]

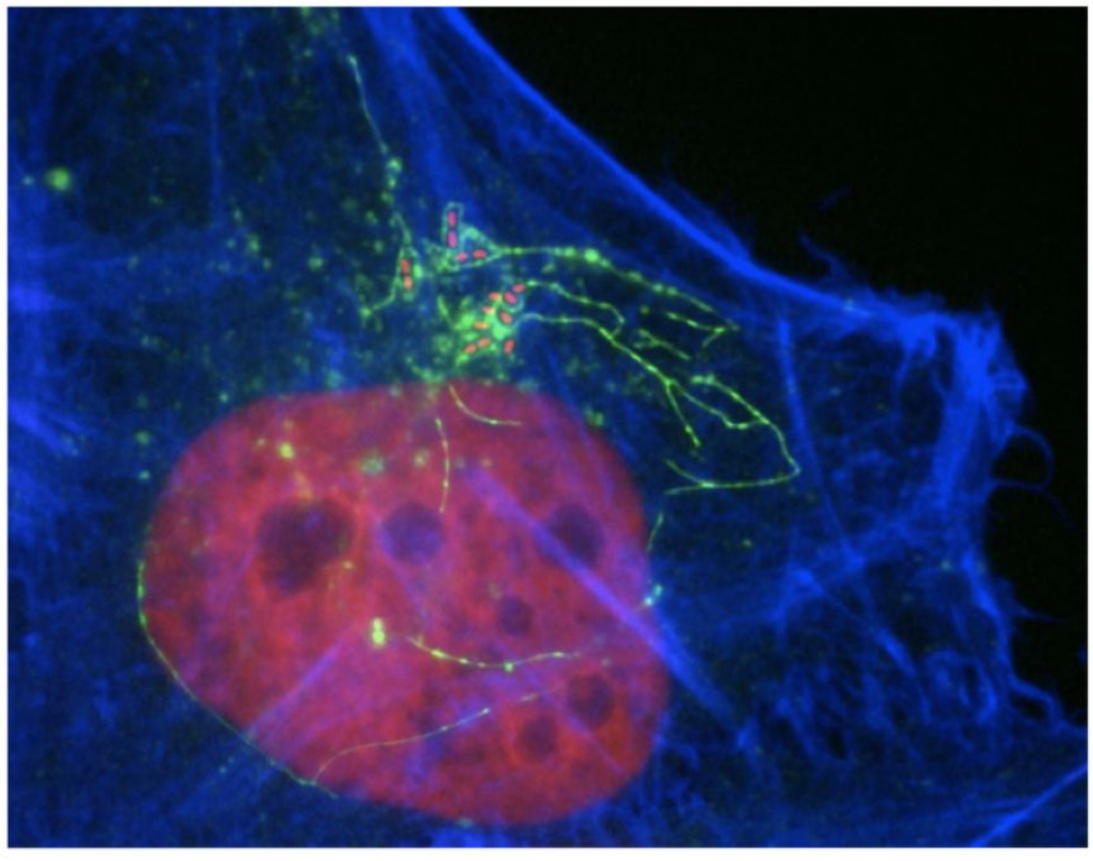
[illegible]
A. Seubert, R. Rappuoli and A. Covacci are at IRIS, Chiron S.r.l., [illegible]

What happens once bacteria are in?



Listeria cytoskeleton

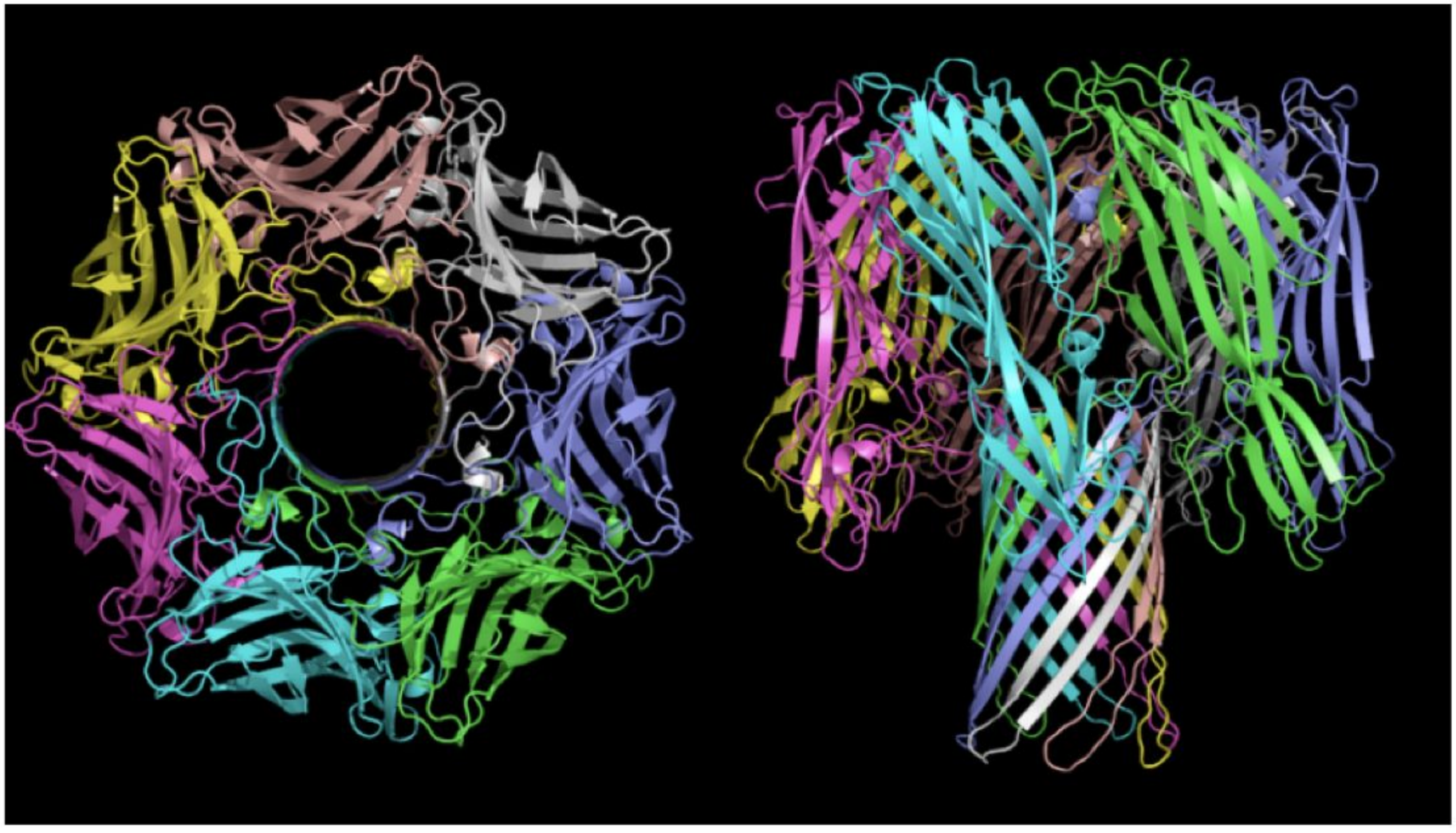




DNA
cytoskeleton
pathogen vacuole

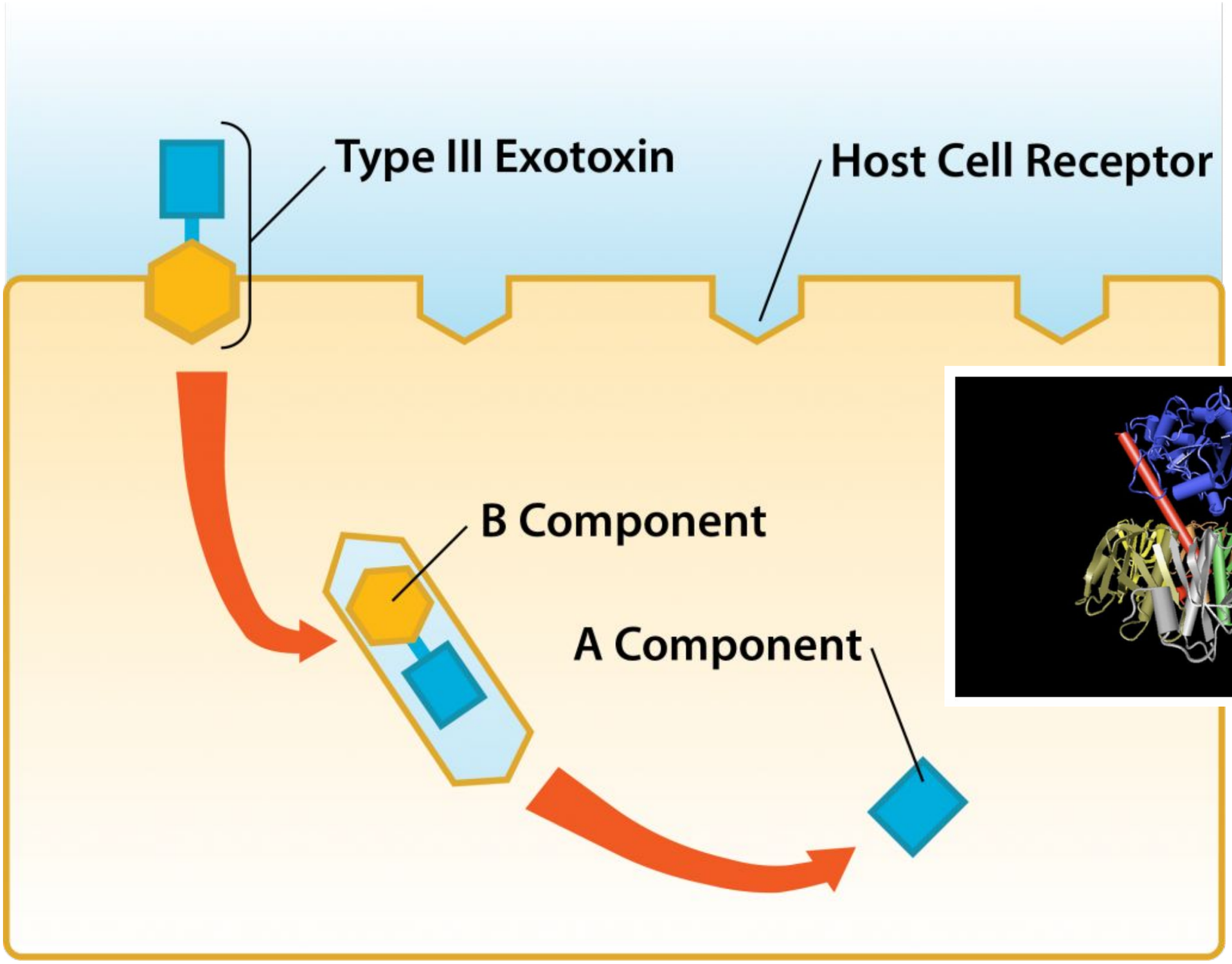
Damage caused by bacteria

- **Direct** – from bacteria action
- **Indirect** – from host response

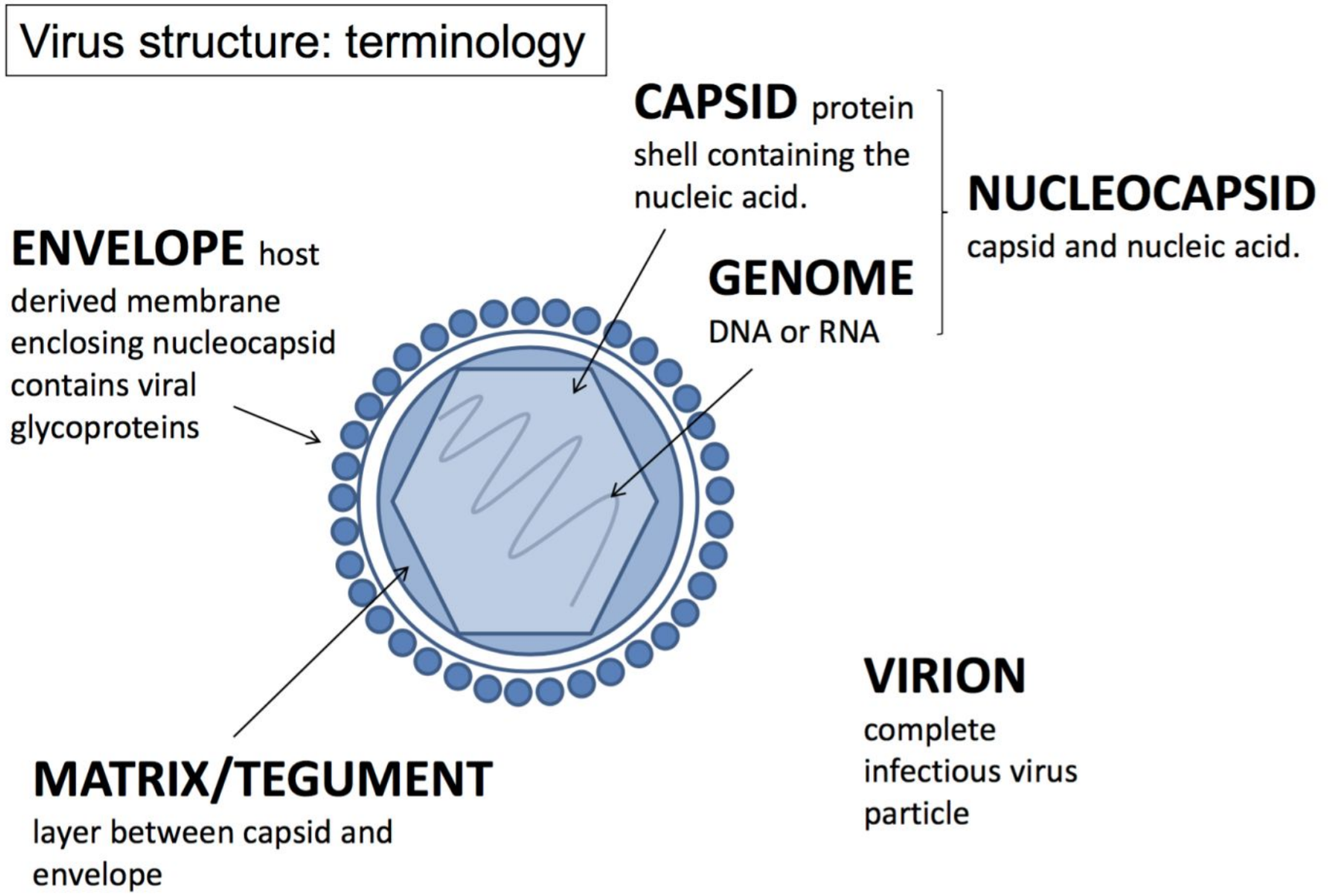


Cytolysin

AB toxins

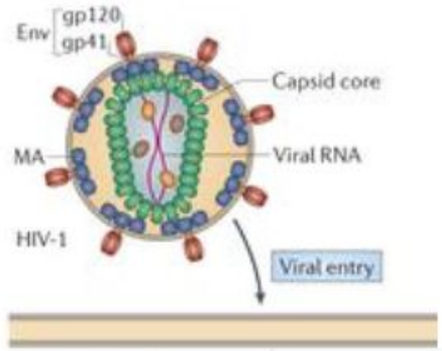

Type III Exotoxin
Host Cell Receptor
B Component
A Component

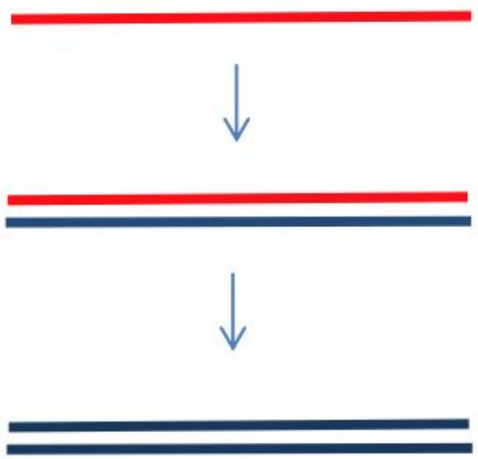
Viruses


Virus structure: terminology
CAPSID protein shell containing the nucleic acid.
NUCLEOCAPSID
capsid and nucleic acid.
GENOME
DNA or RNA
ENVELOPE host derived membrane enclosing nucleocapsid contains viral glycoproteins
MATRIX/TEGUMENT
layer between capsid and envelope
VIRION
complete infectious virus particle

Retroviruses - HIV

Replication enzymes
contained in capsid

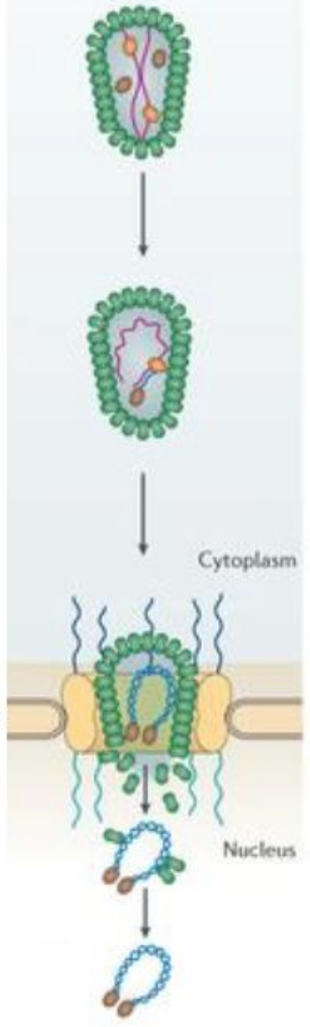




RNA dependent
DNA synthesis

DNA dependent
DNA synthesis

Reverse
transcriptase

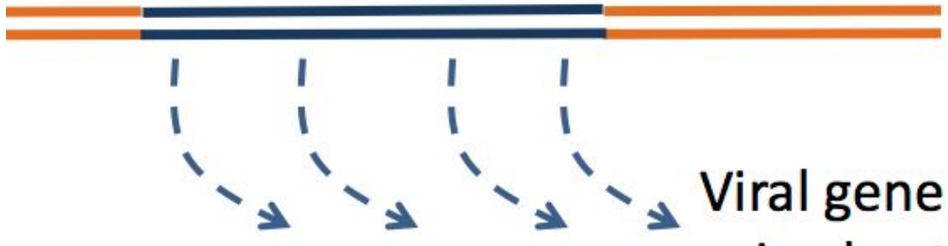


Cytoplasm

Nucleus

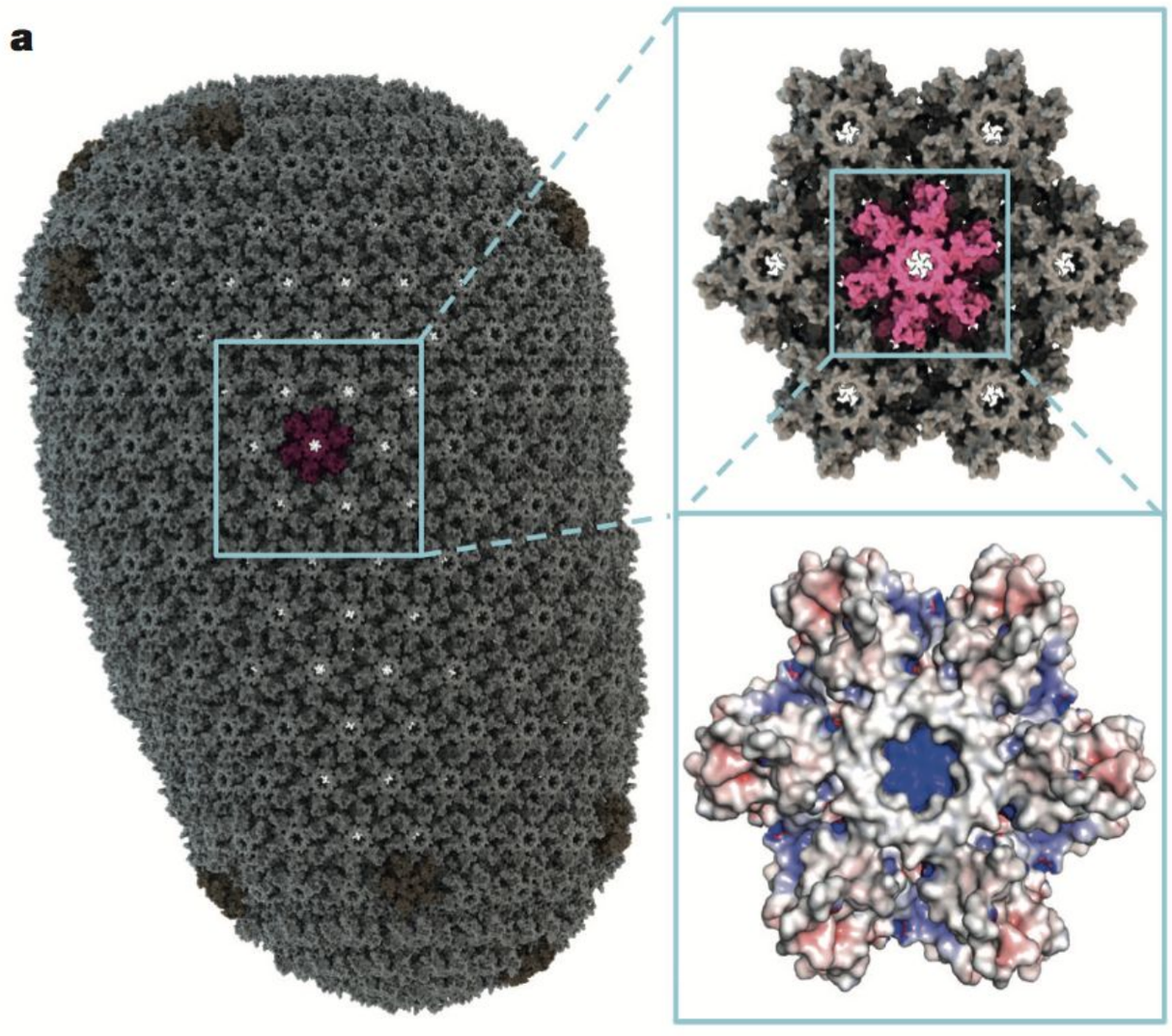
Integration into
host genome

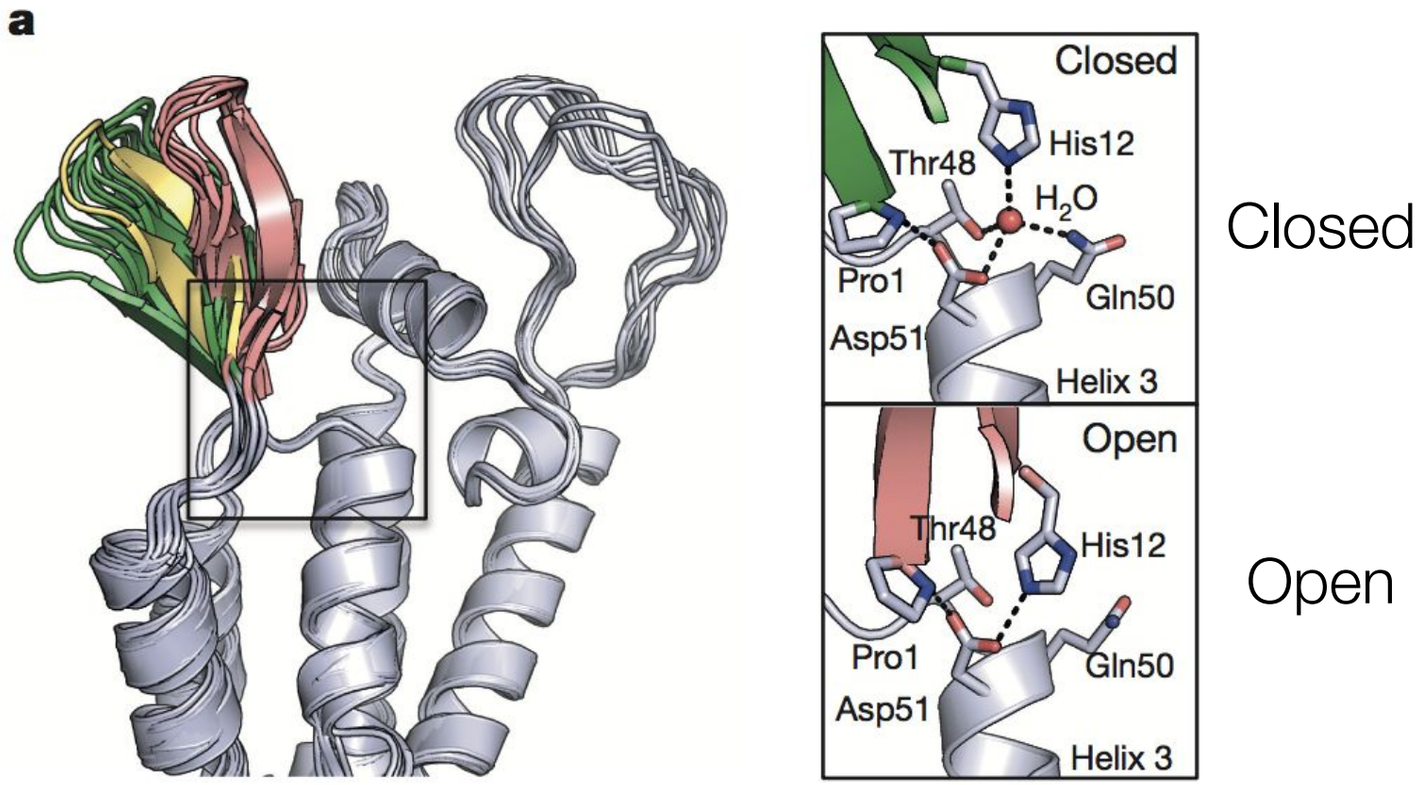
Integrase



Viral gene expression
using host machinery

HIV capsid

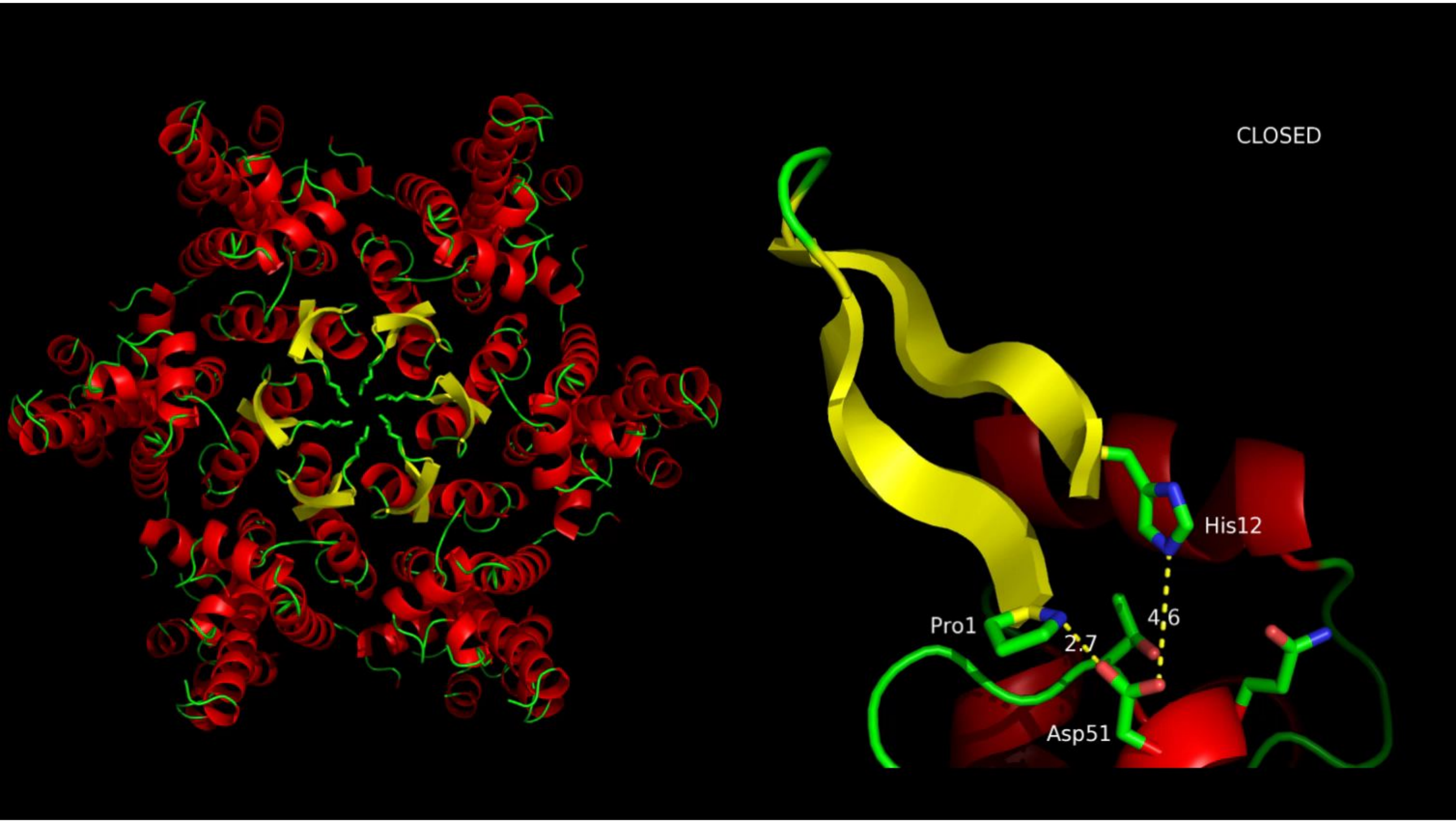


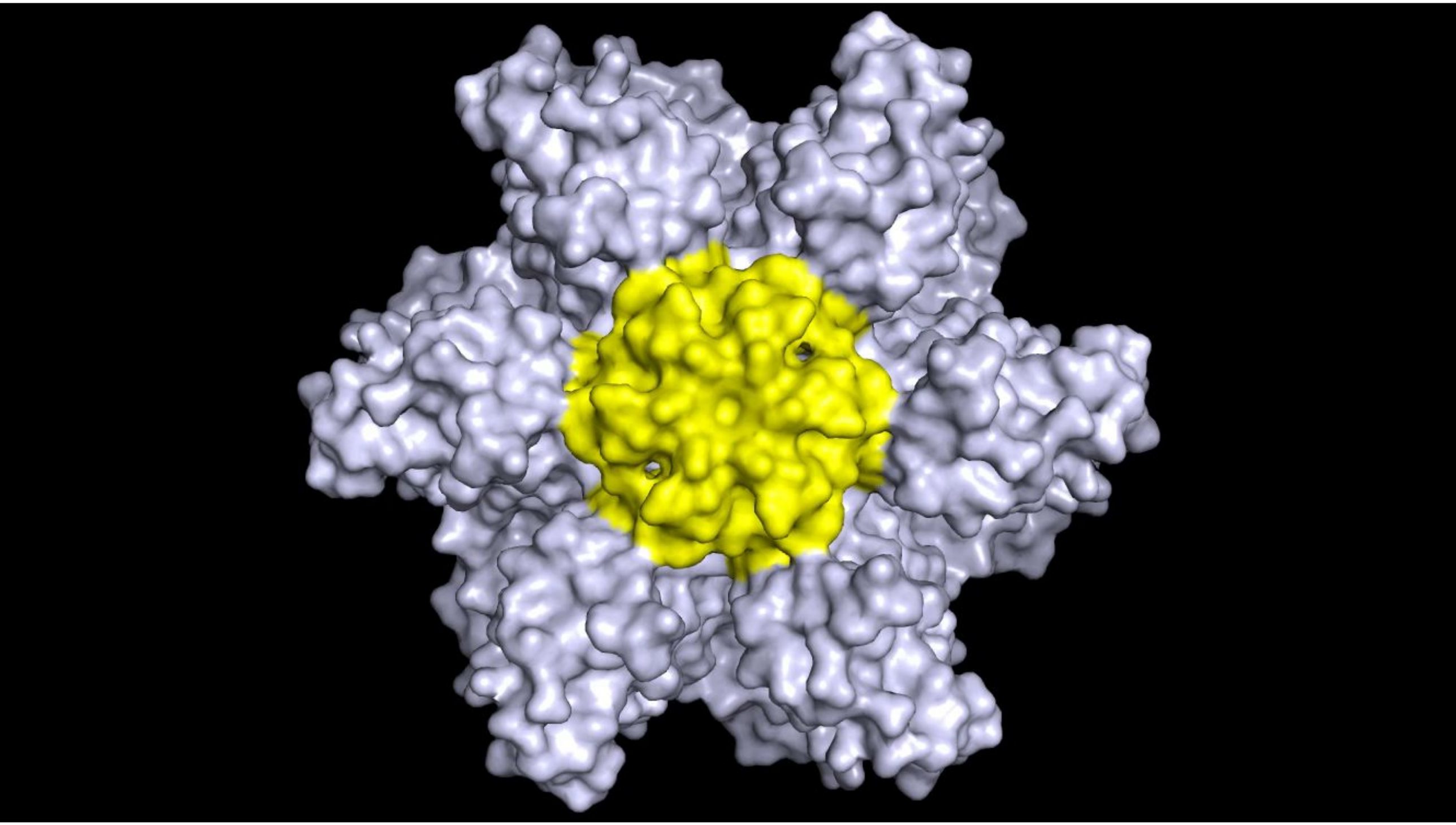


Closed

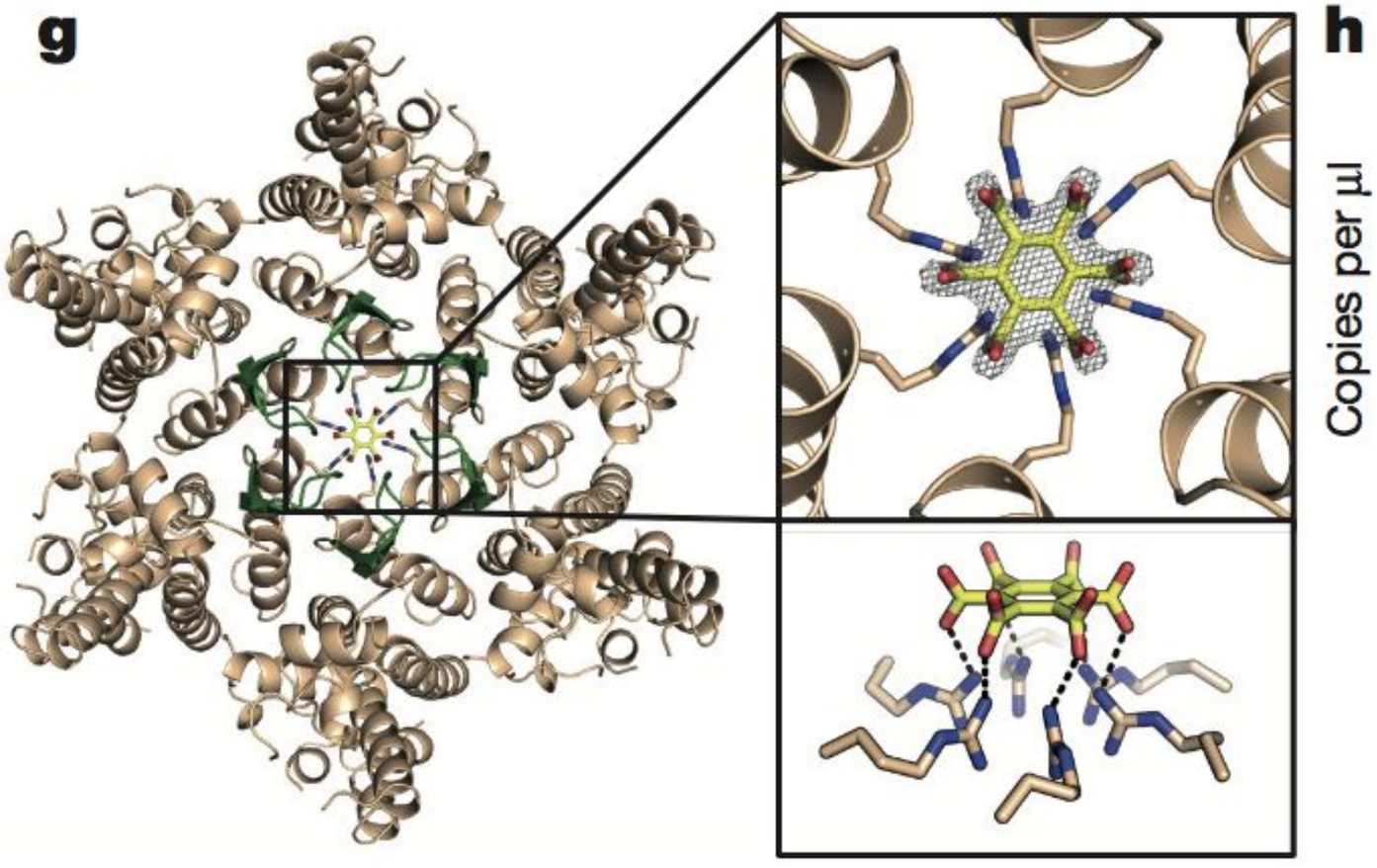
Open

Beta-hairpin can adopt alternate conformations that differ by up to 15 Å.


CLOSED
His12
Pro1
2.7
4.6
Asp51



HIV-1 reverse transcription is inhibited by blockade of the capsid pore

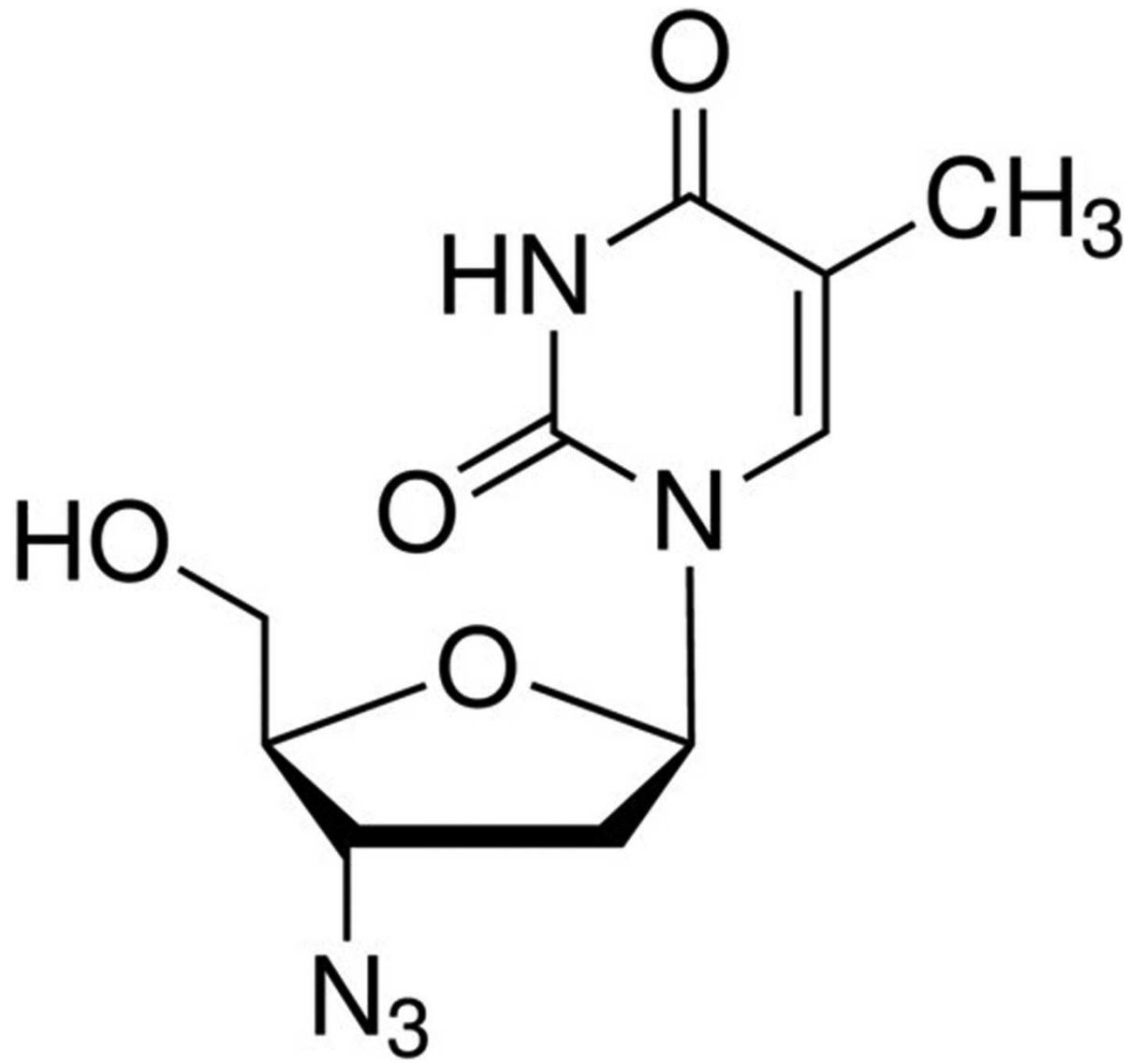


$CA_{hexamer}$ crystal structure in complex with hexacarboxybenzene, which is co-ordinated by R18

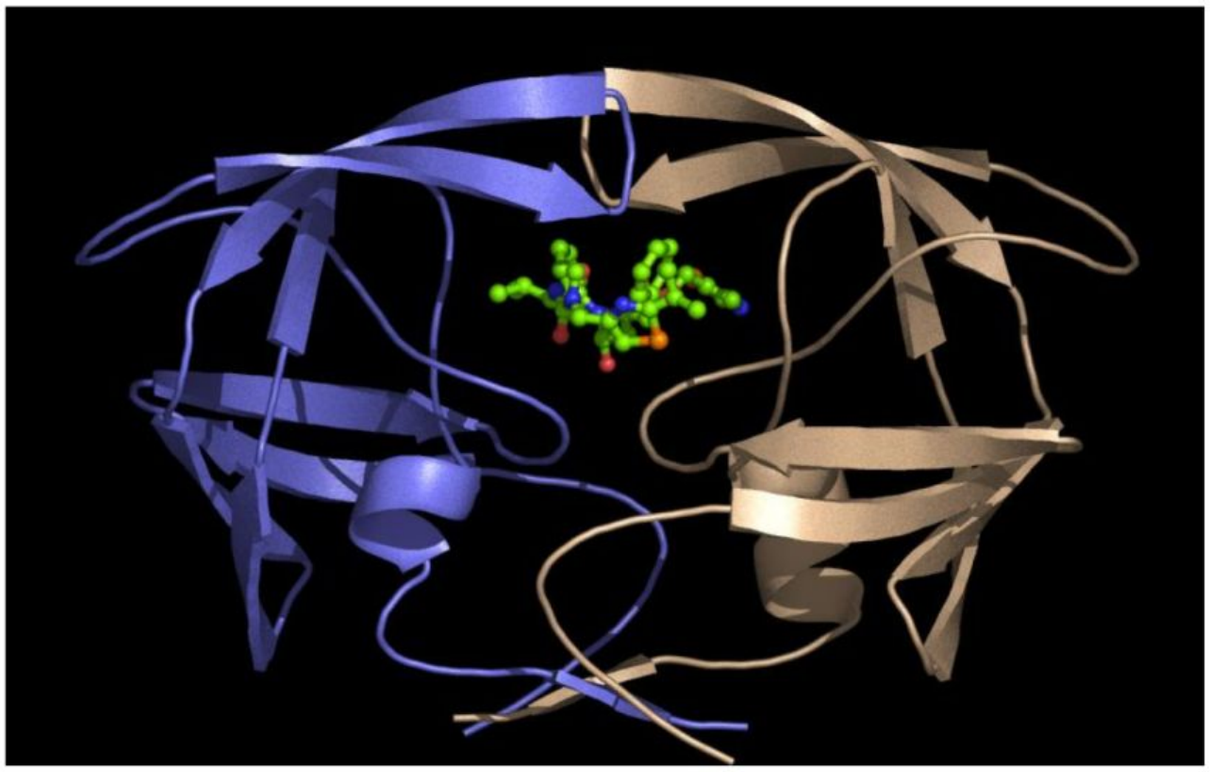
Current approach to AIDS treatment

- Nucleoside Reverse Transcriptase Inhibitors
- Protease Inhibitors
- Fusion Inhibitors
- Highly Active Antiretroviral Therapy
- Non-Nucleoside Reverse Transcriptase Inhibitors

AZT



Blocking assembly: protease inhibitors

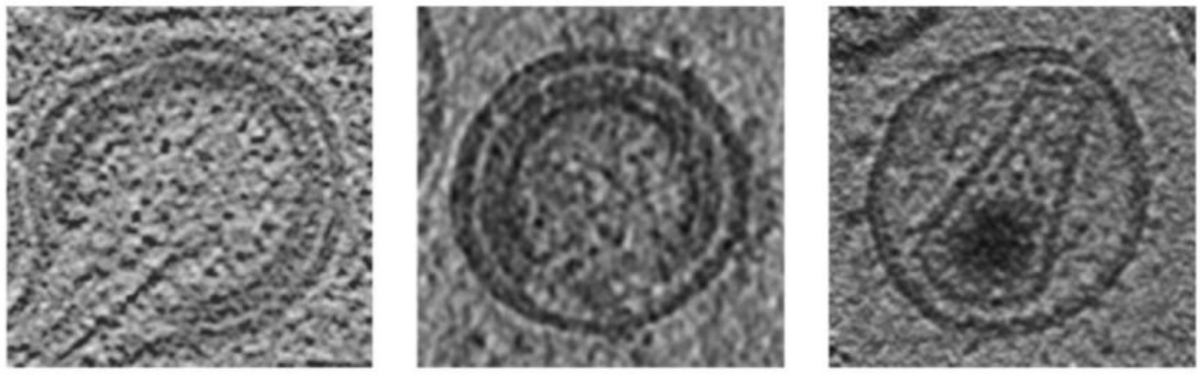


<u>HIV protease</u>

Homo-dimeric aspartyl protease

Encoded by pol gene

Peptide mimics of cleavage sites are inhibitors



Cleaves polyprotein to produce capsid components