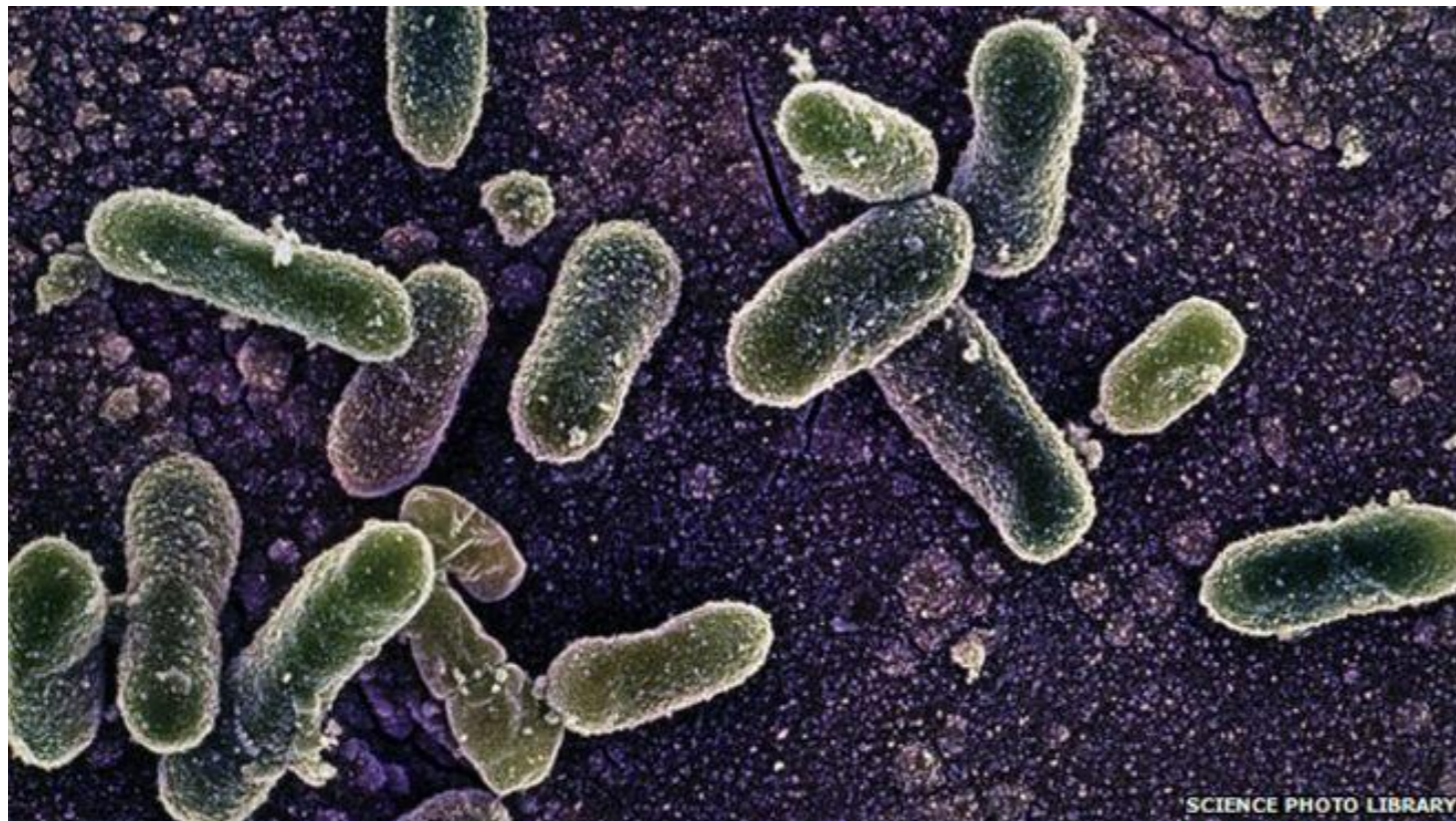
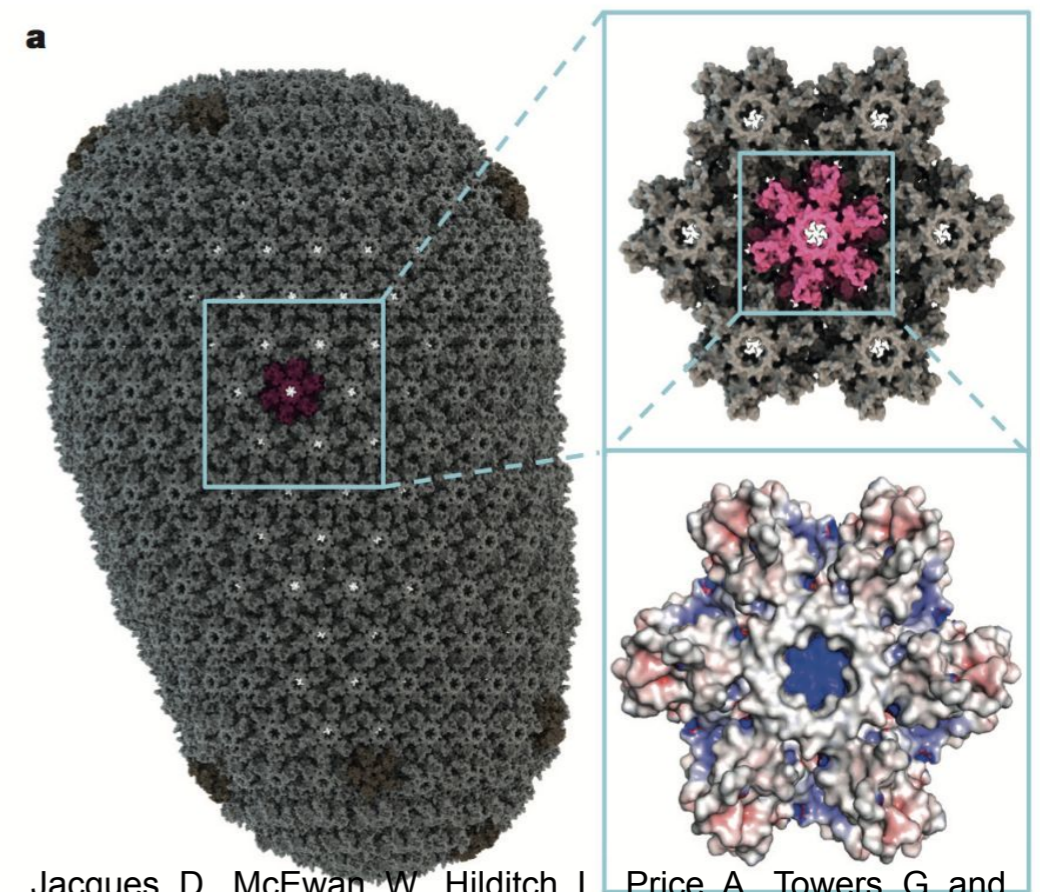


# Pathogenesis of bacteria and viruses



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



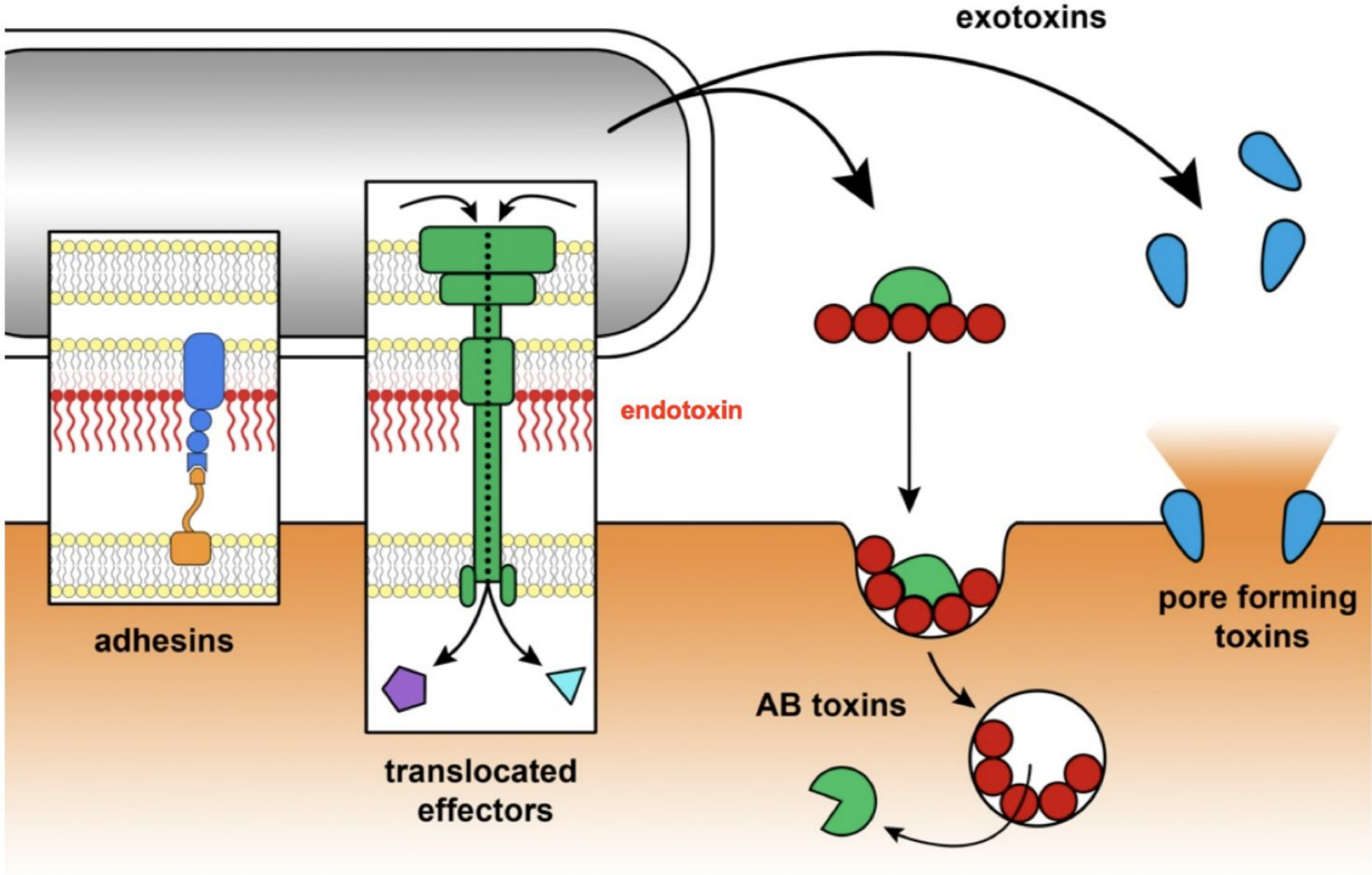
Jacques, D., McEwan, W., Hilditch, L., Price, A., Towers, G. and

James, L. (2016). HIV-1 uses dynamic capsid pores to import nucleotides and fuel encapsidated DNA synthesis. *Nature*,

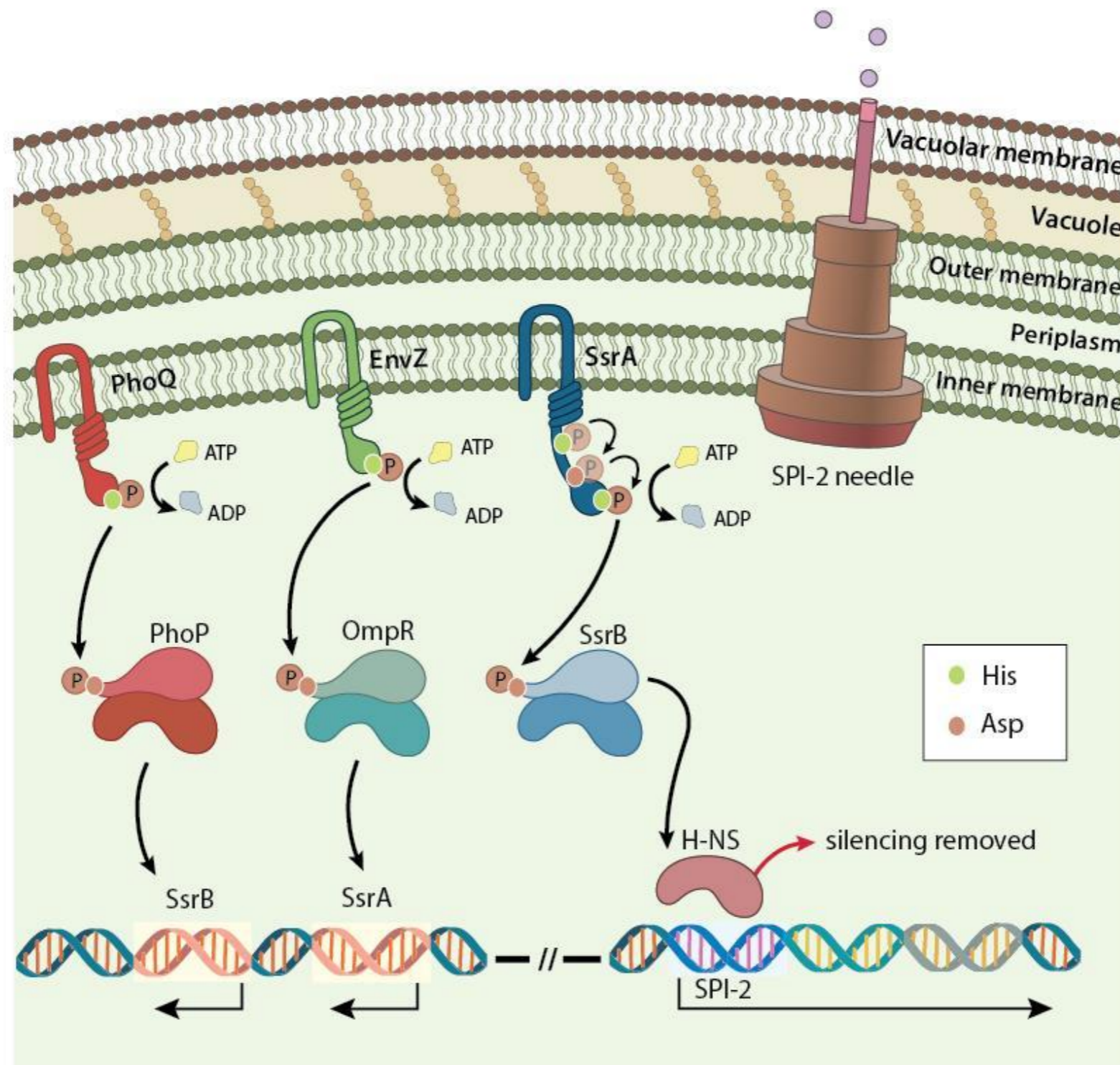


# Bacteria

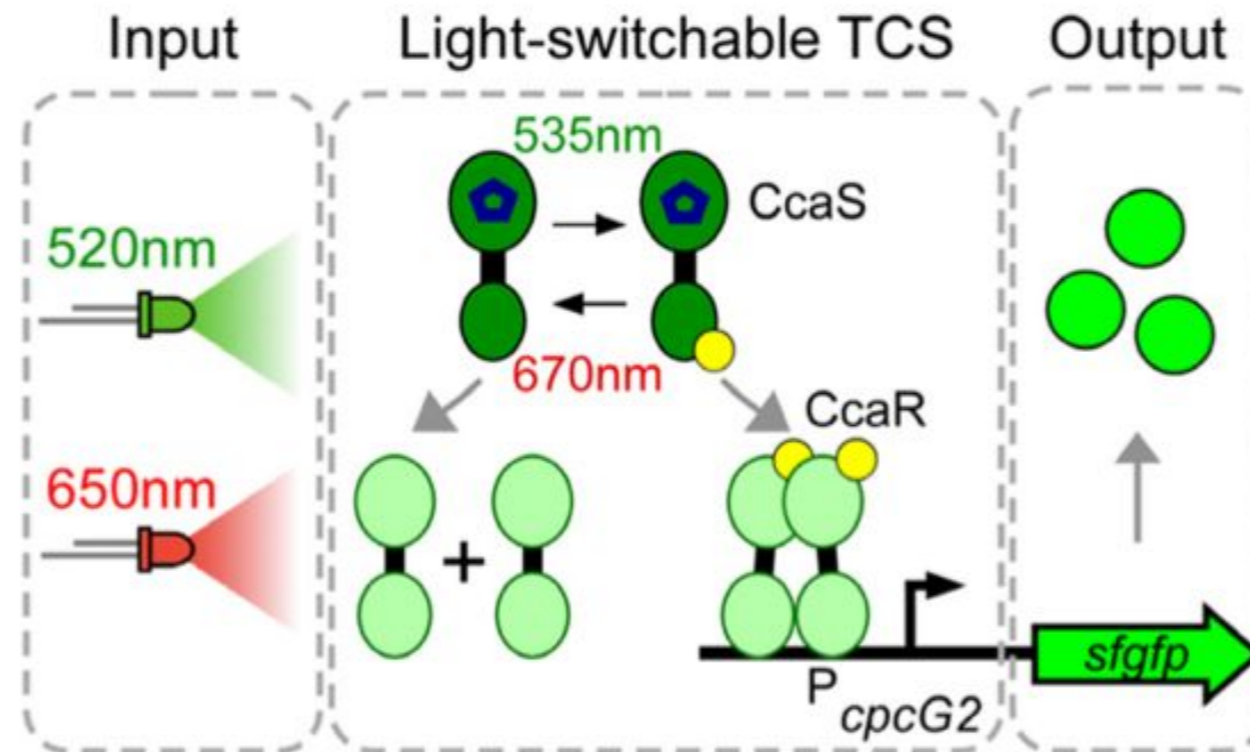
Virulence factors



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



# A bit of synthetic biology...

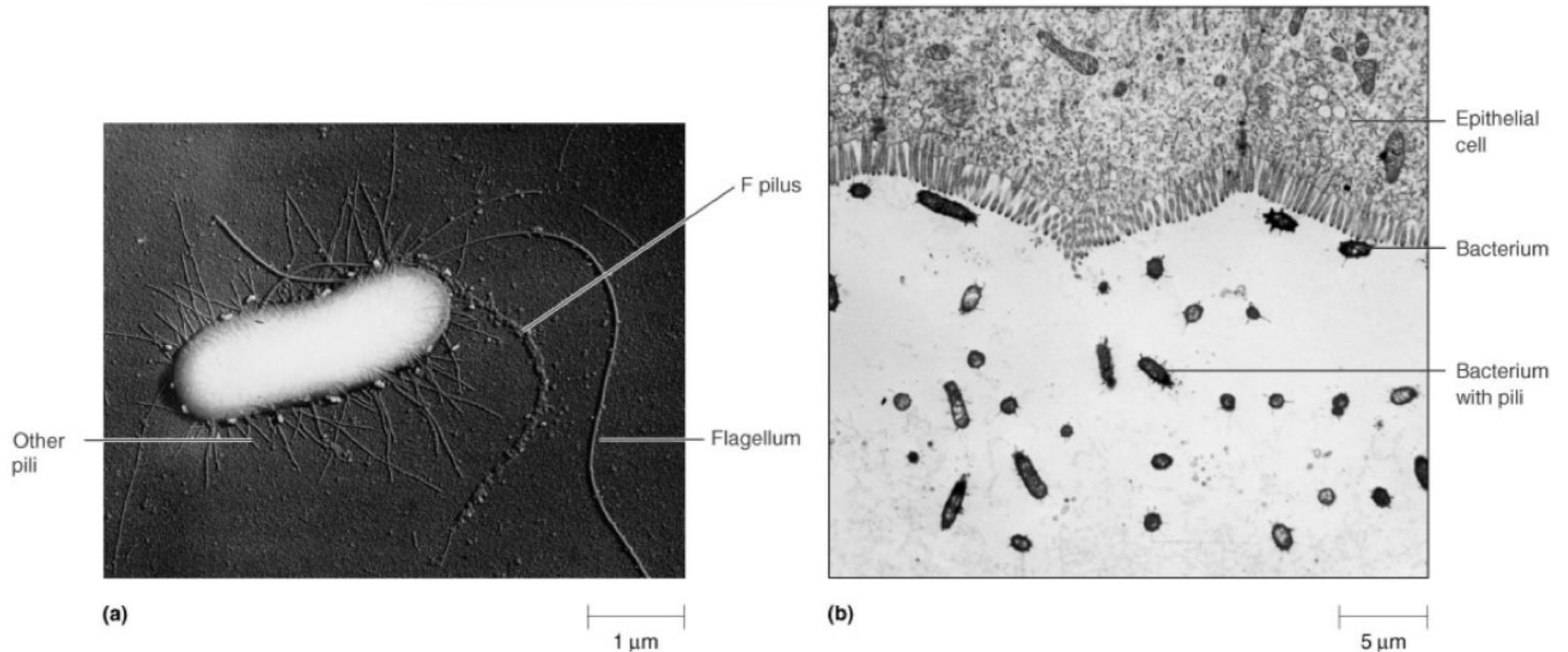


Schmidl, S., Sheth, R., Wu, A. and Tabor, J. (2014). Refactoring and Optimization of Light-Switchable *Escherichia coli* Two-Component Systems. *ACS Synthetic Biology*, 3(11), pp.820-831.



# Key events of pathogen-host interaction

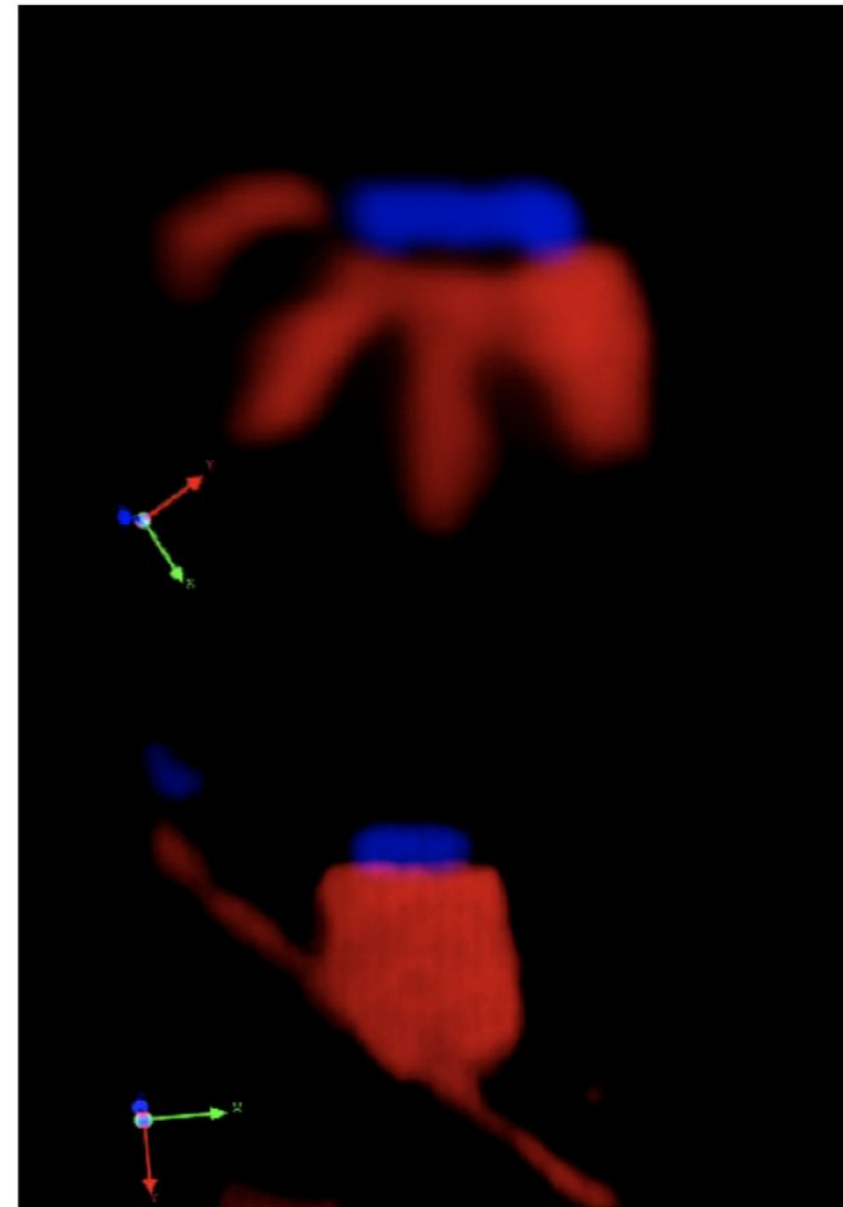
1. Colonisation - invasion
2. Multiplication
3. Transmission
4. Damage



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



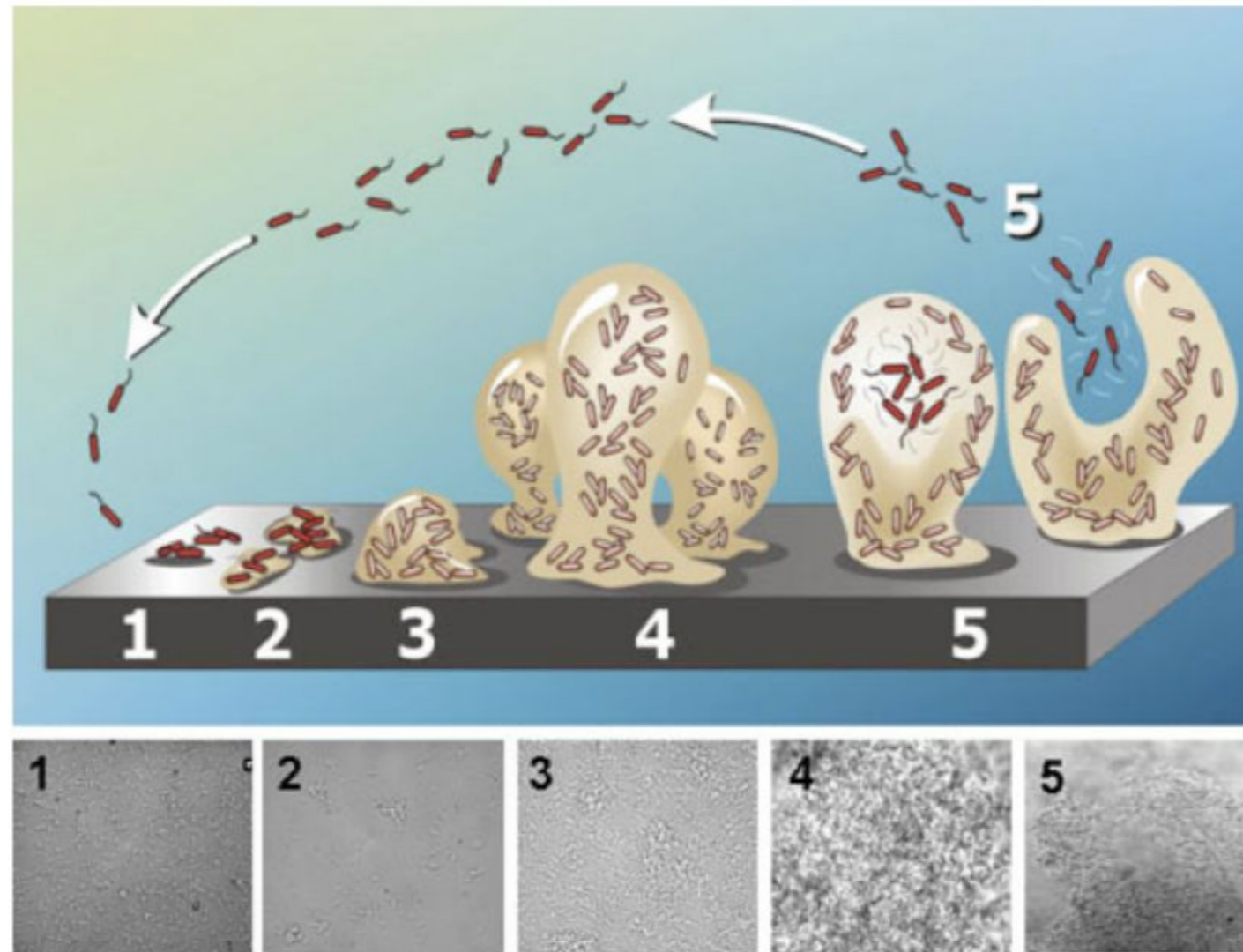
scanning electron microscopy



cytoskeleton  
enteropathogenic *E. coli*

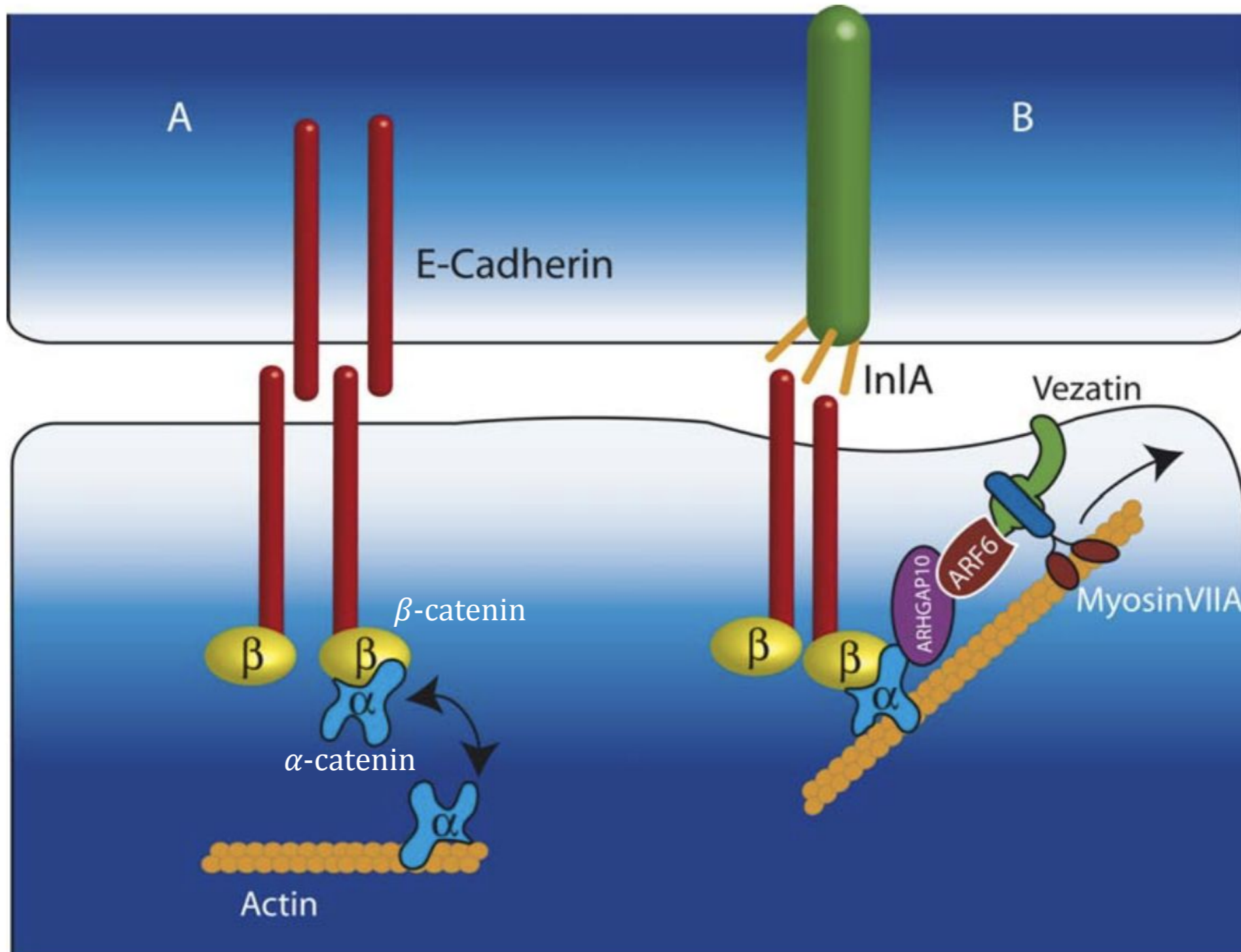
Enteropathogenic *E. coli* build specialised structures for adhesion

# Biofilms

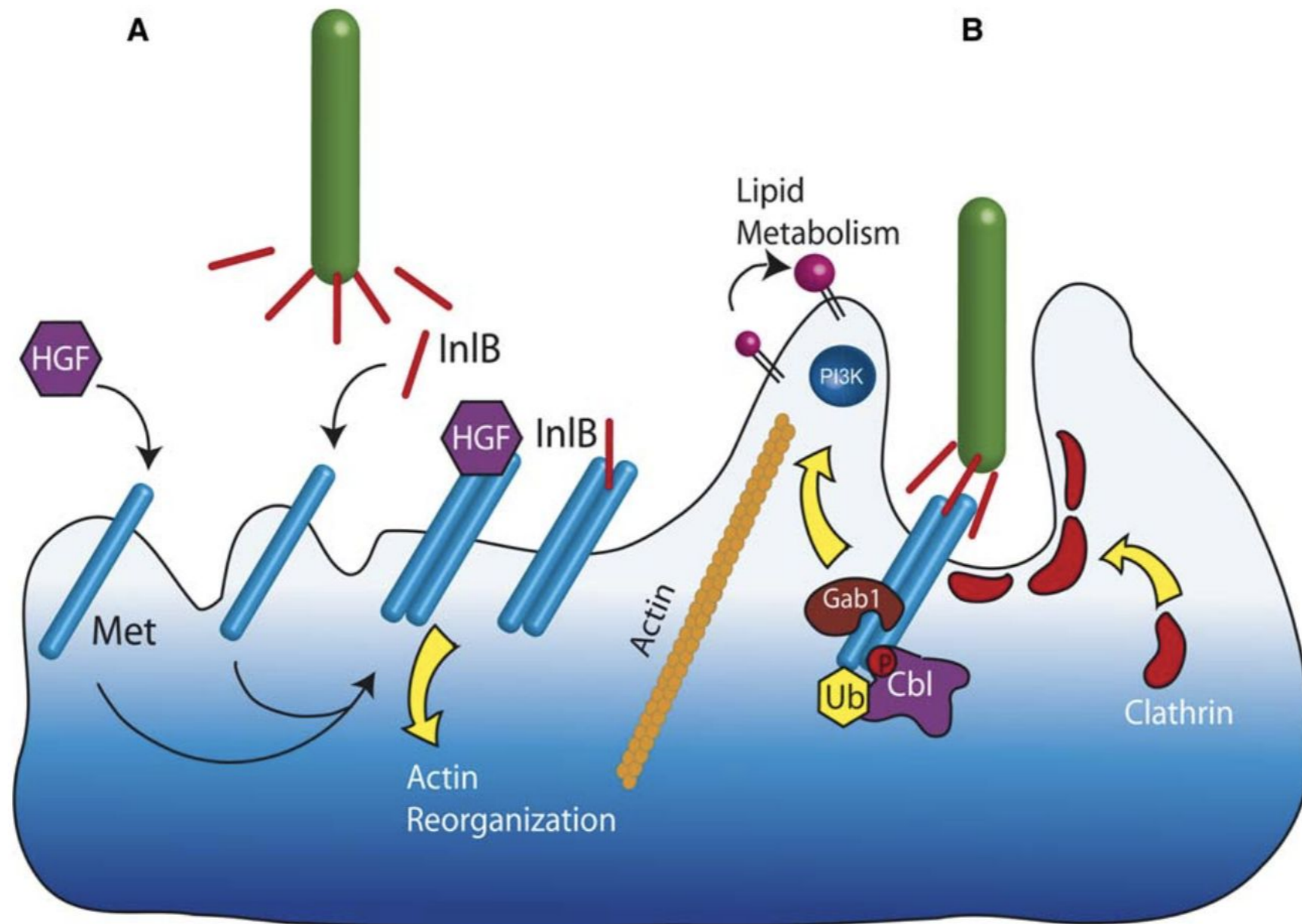


# Invasion

“Zipper mechanism”. *Listeria* invading non-phagocytic cells.



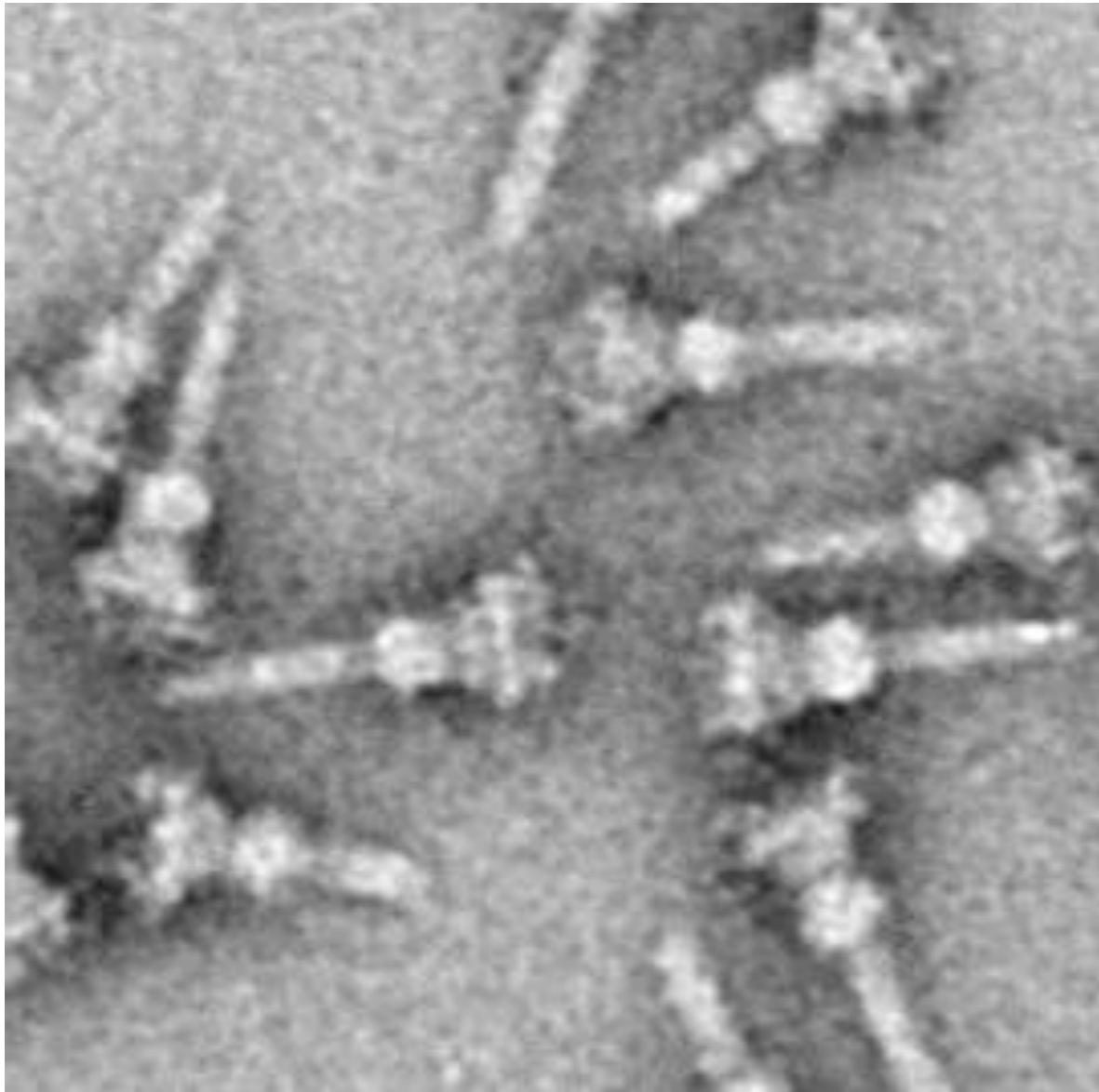




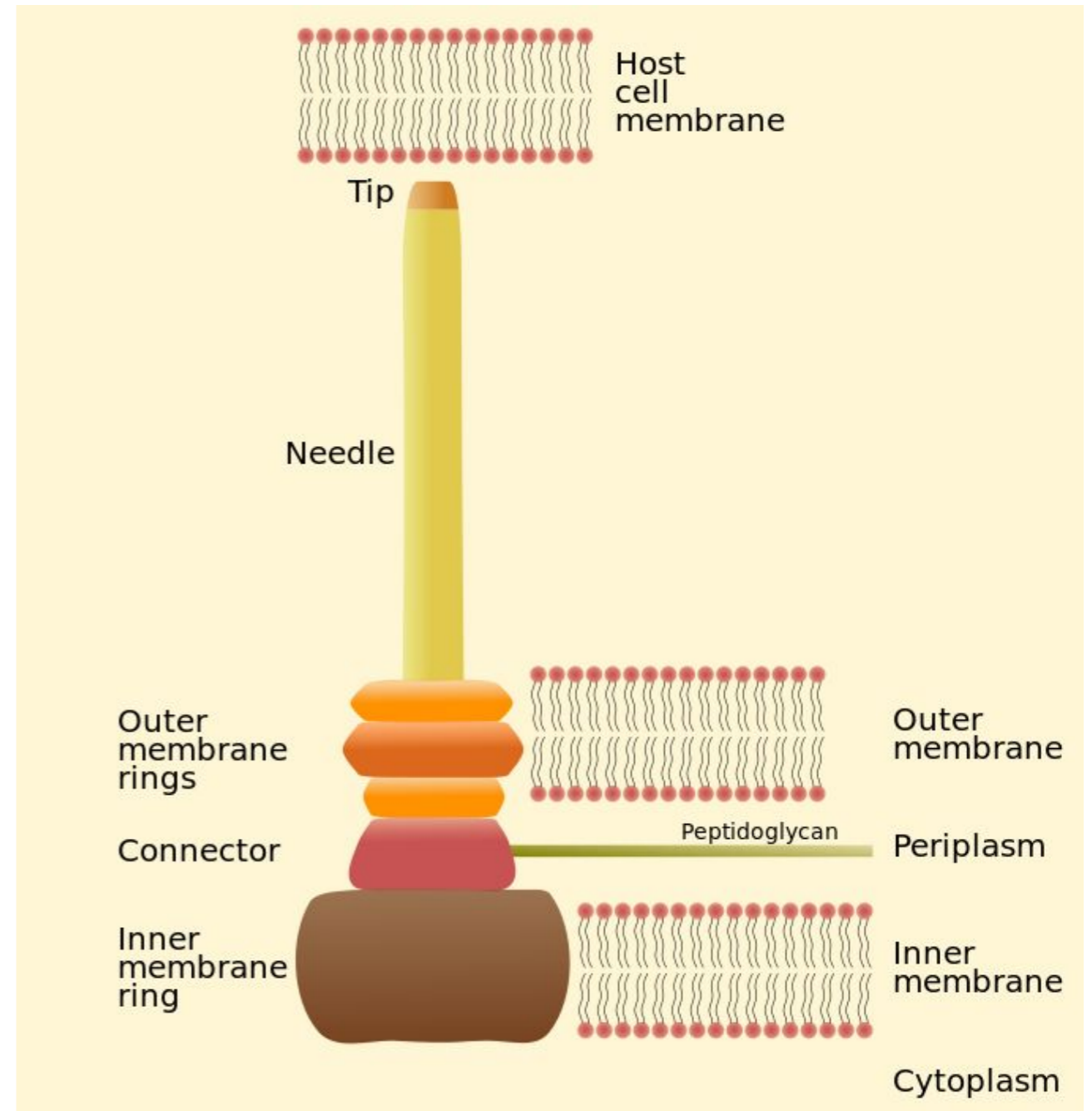
Bonazzi, M. and Cossart, P. (2006). Bacterial entry into cells: A role for the endocytic machinery. *FEBS Letters*, 580(12), pp.2962-2967.

# “Trigger mechanism”. *Salmonella*.

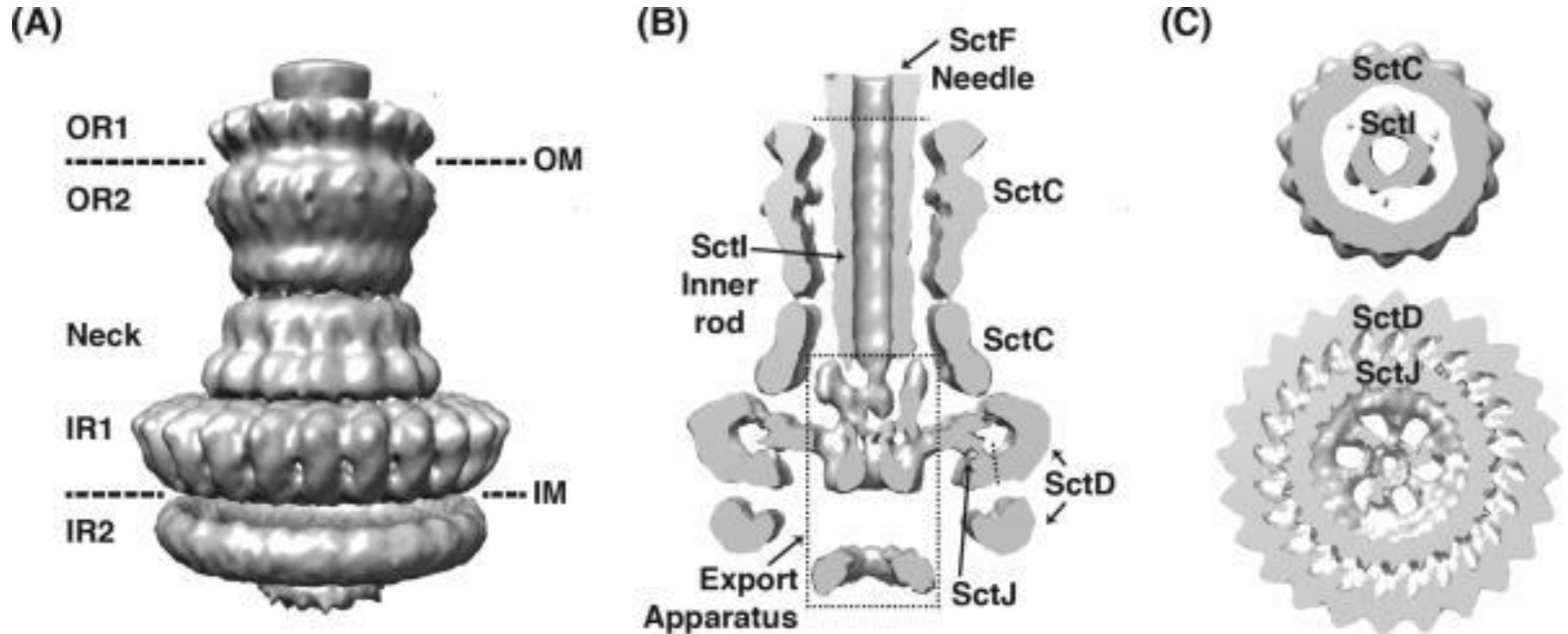
## Type III Secretion System



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.



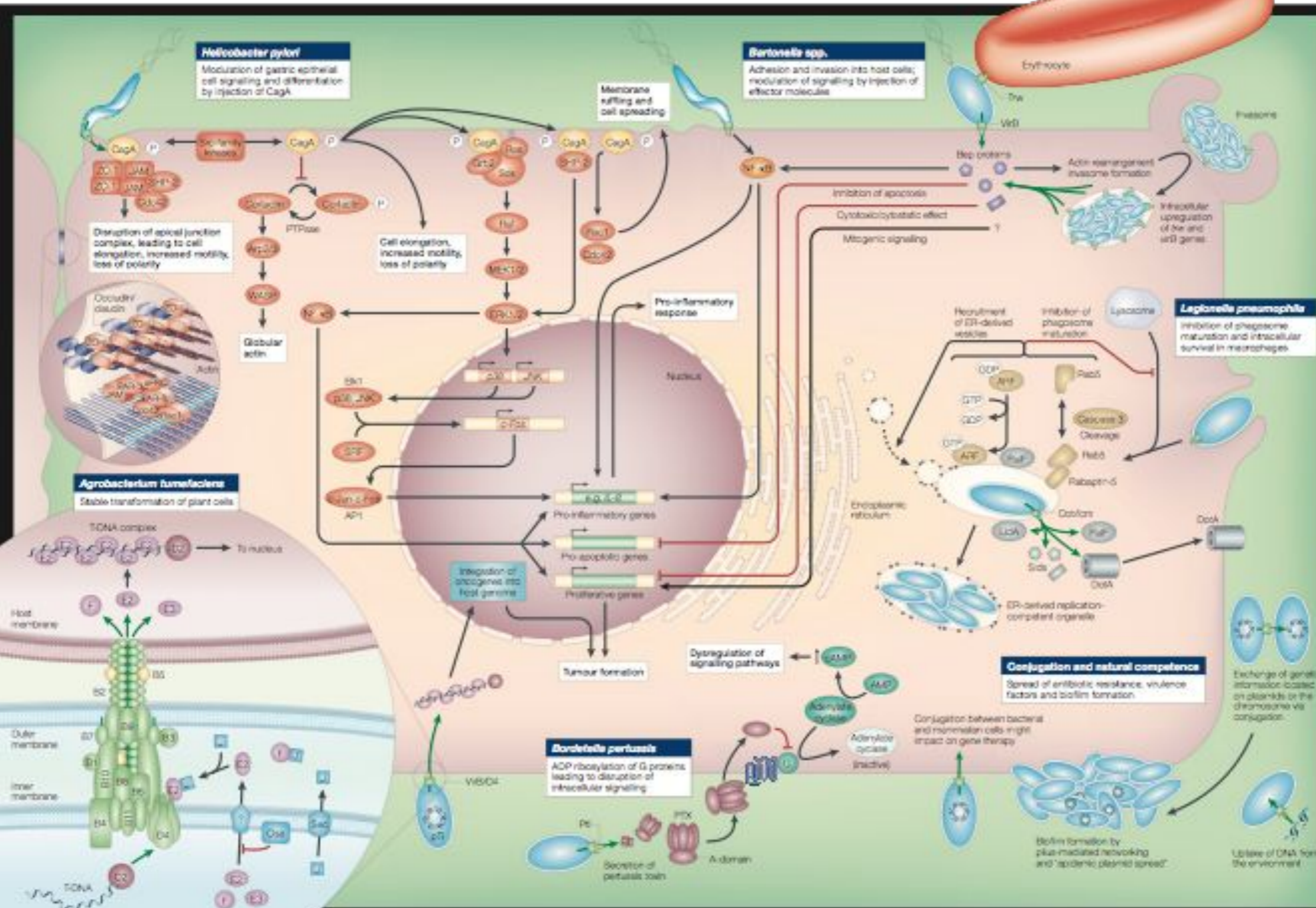
# Type IV secretion

Anja Seubert, Rino Rappuoli and Antonello Covacci

# CHIRON

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 materials 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 summarises 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 Tjv system, where extensive gene duplication creates variant plus subunits, is necessary for colonization of erythrocytes. The Vrb system and its secreted *Bartonella* effector proteins (Bops) are believed to be responsible for most of the cellular effects of the interaction of *Bartonella* with host endothelial cells: actin rearrangements, resulting in invagination 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 systems 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 (Sida substrate of Icm/Dot transporters) 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.

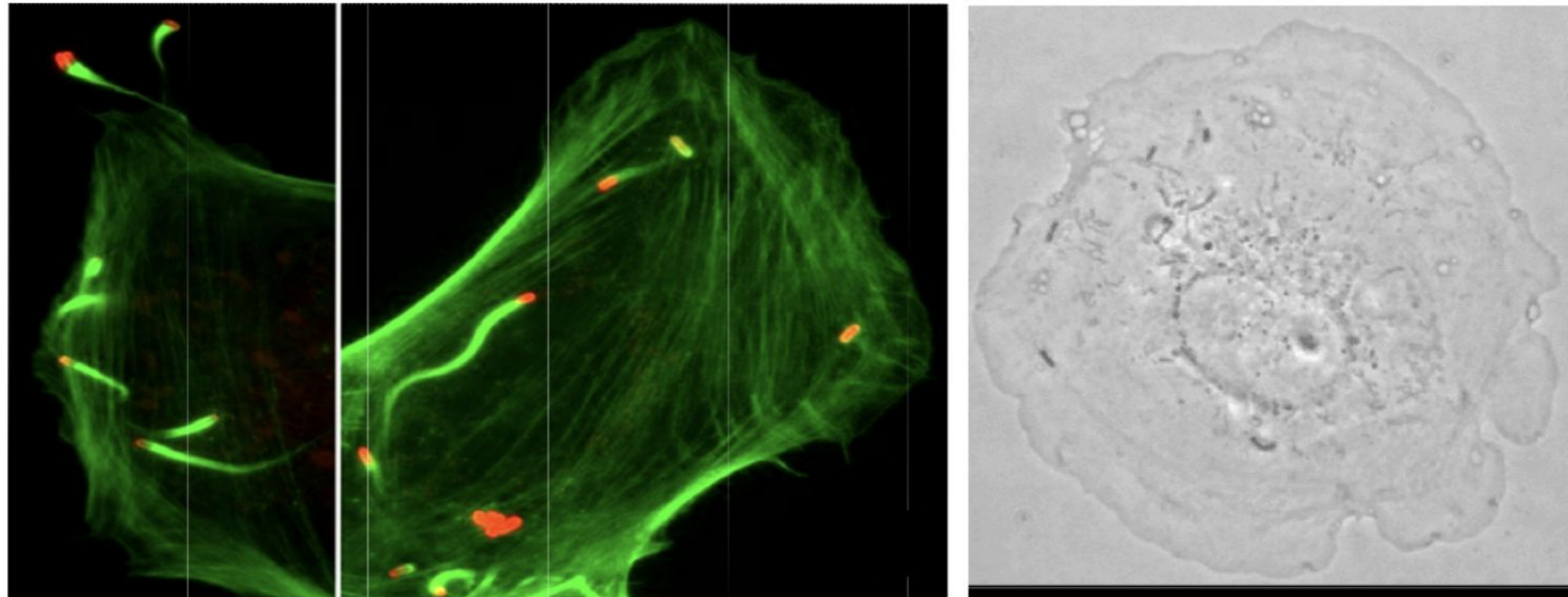
**Agrobacterium tumefaciens**  
The VirB/D4 system in the plant pathogen *A. tumefaciens* is the prototypical T4SS. *A. tumefaciens* translocates oncogenic single-stranded DNA (T-DNA) into a variety of dicotyledonous plants, causing tumours known as crown galls. The T-DNA is translocated as a nucleoprotein complex with VirD2, which, along with VirE1, 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 VirE2, which is also of unknown function.

**Bordetella pertussis**  
The Pil 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.

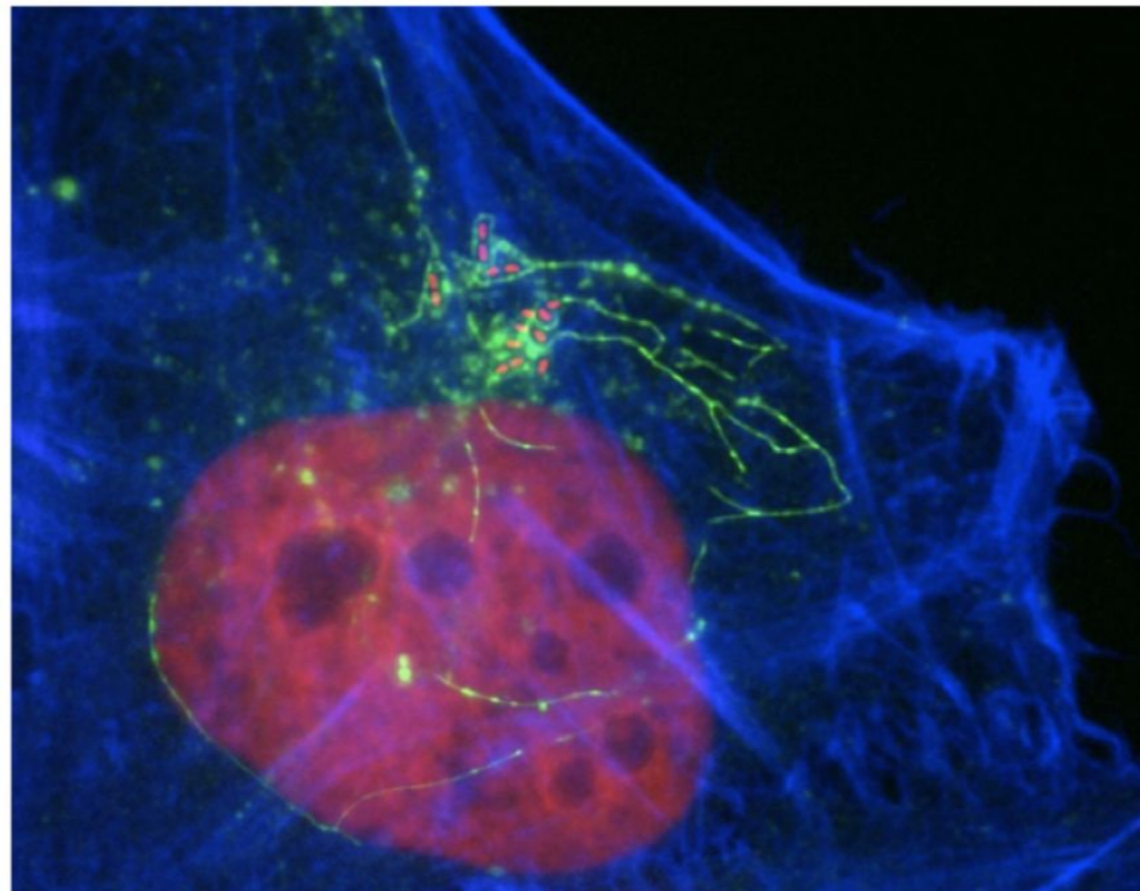
**FURTHER READING**  
Barni, E.L. Type IV secretion systems. *Curr Opin Microbiol* 8, 26-34 (2005).  
Covacci, A. & Rappuoli, R. The secretory type IV secretion system. *Nature Rev Microbiol* 1, 127-138 (2003).  
Dworkin, D. Secretion systems in Gram-negative bacteria. *Nature Rev Microbiol* 1, 127-138 (2003).  
Dworkin, D. Secretion systems in Gram-negative bacteria. *Nature Rev Microbiol* 1, 127-138 (2003).  
Dworkin, D. Secretion systems in Gram-negative bacteria. *Nature Rev Microbiol* 1, 127-138 (2003).



# What happens once bacteria are in?



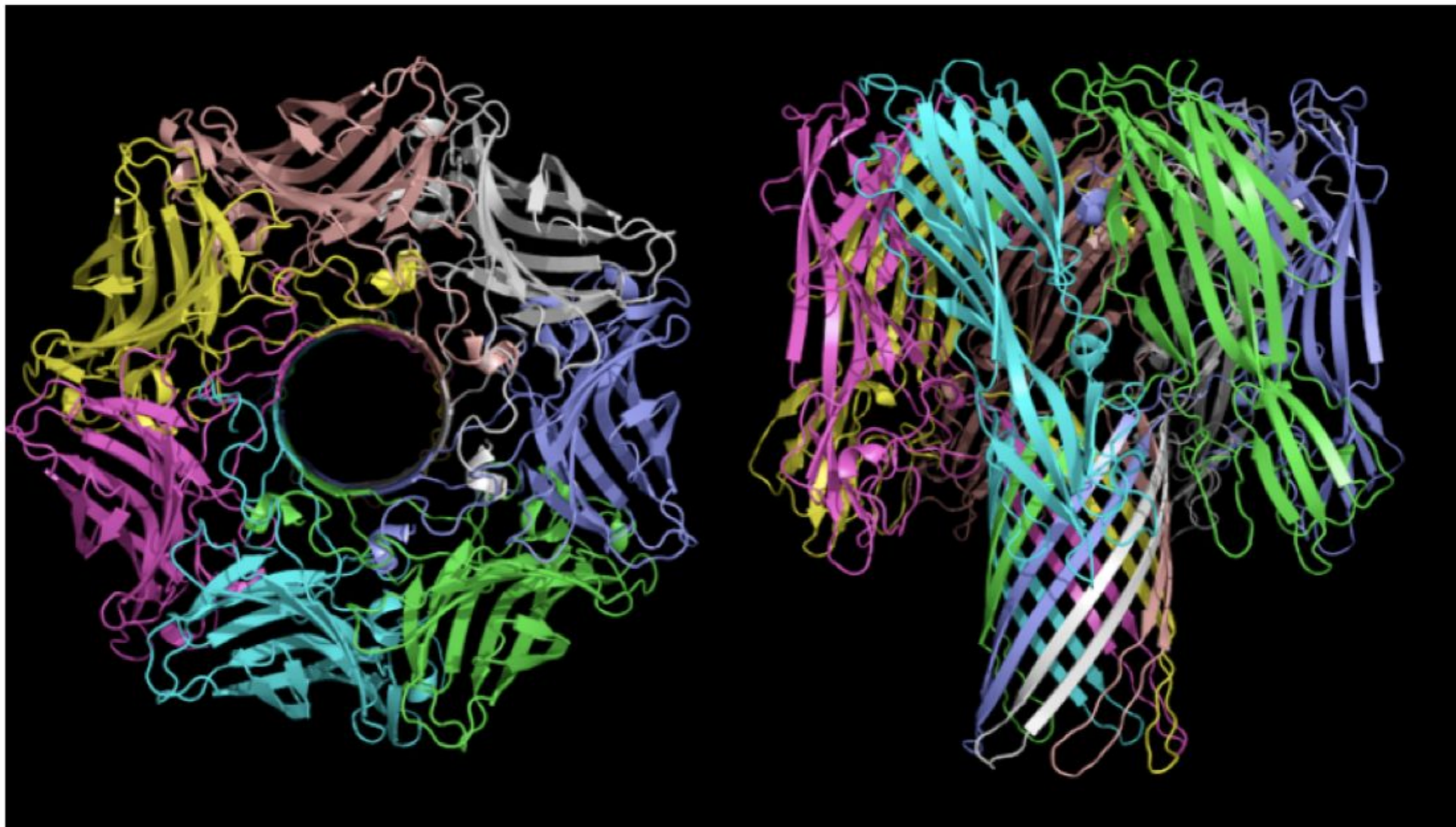
*Listeria* cytoskeleton



DNA  
cytoskeleton  
pathogen vacuole

# Damage caused by bacteria

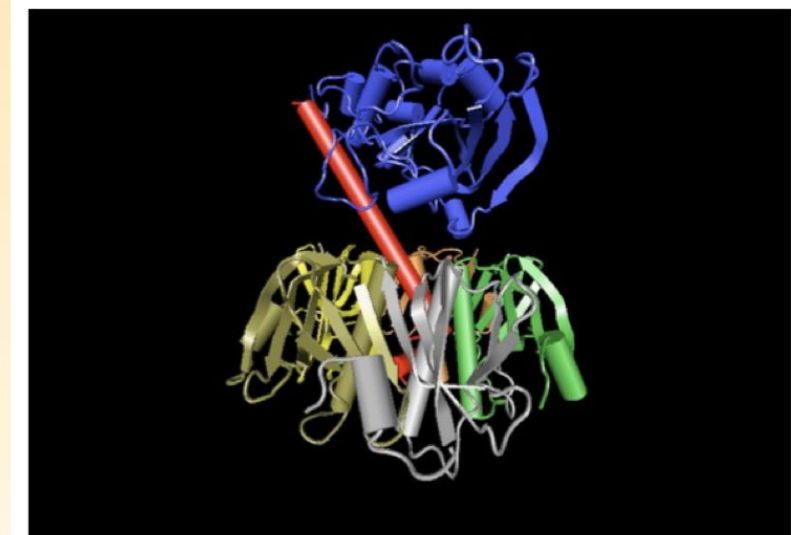
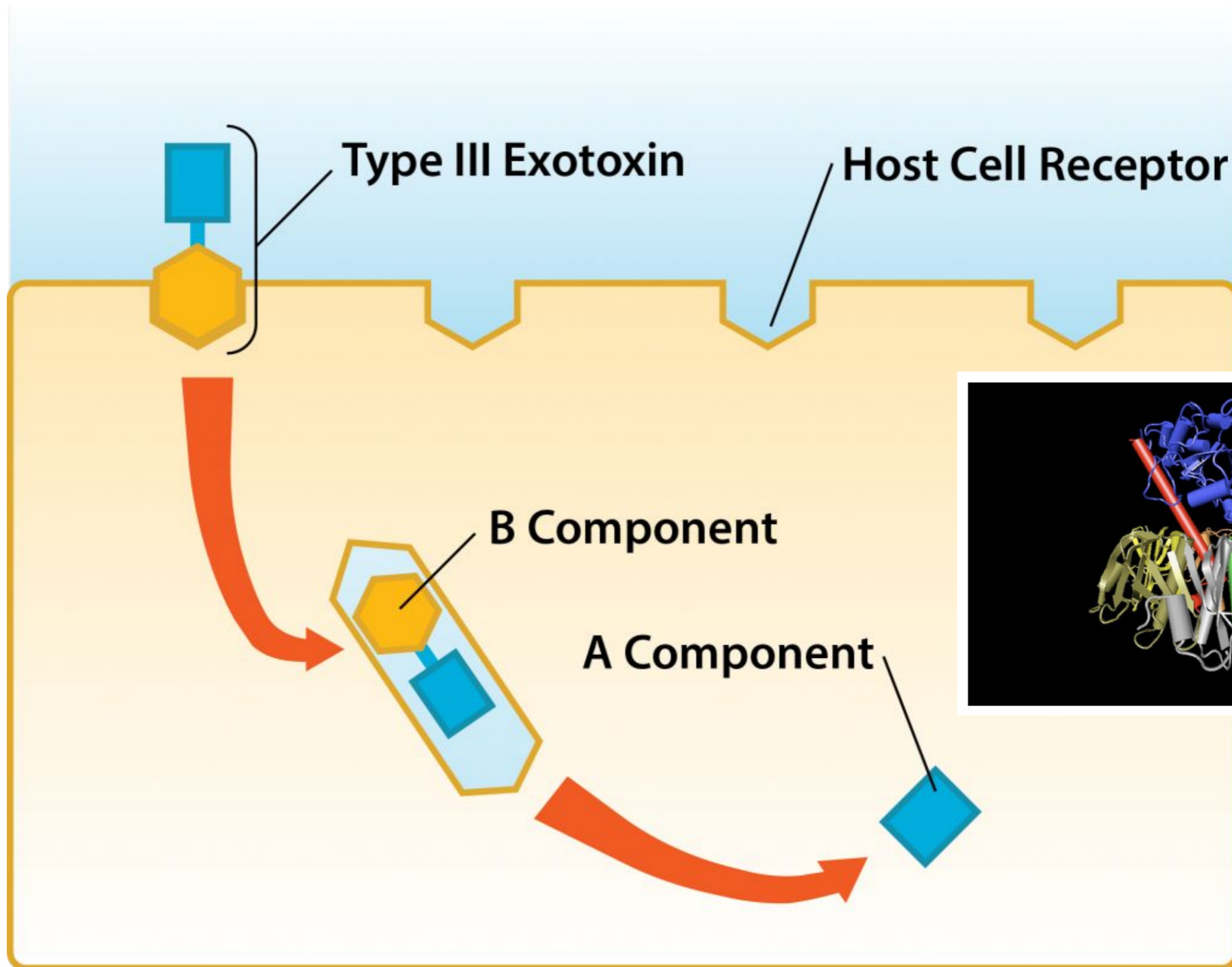
- **Direct** – from bacteria action
- **Indirect** – from host response



Cytolysin

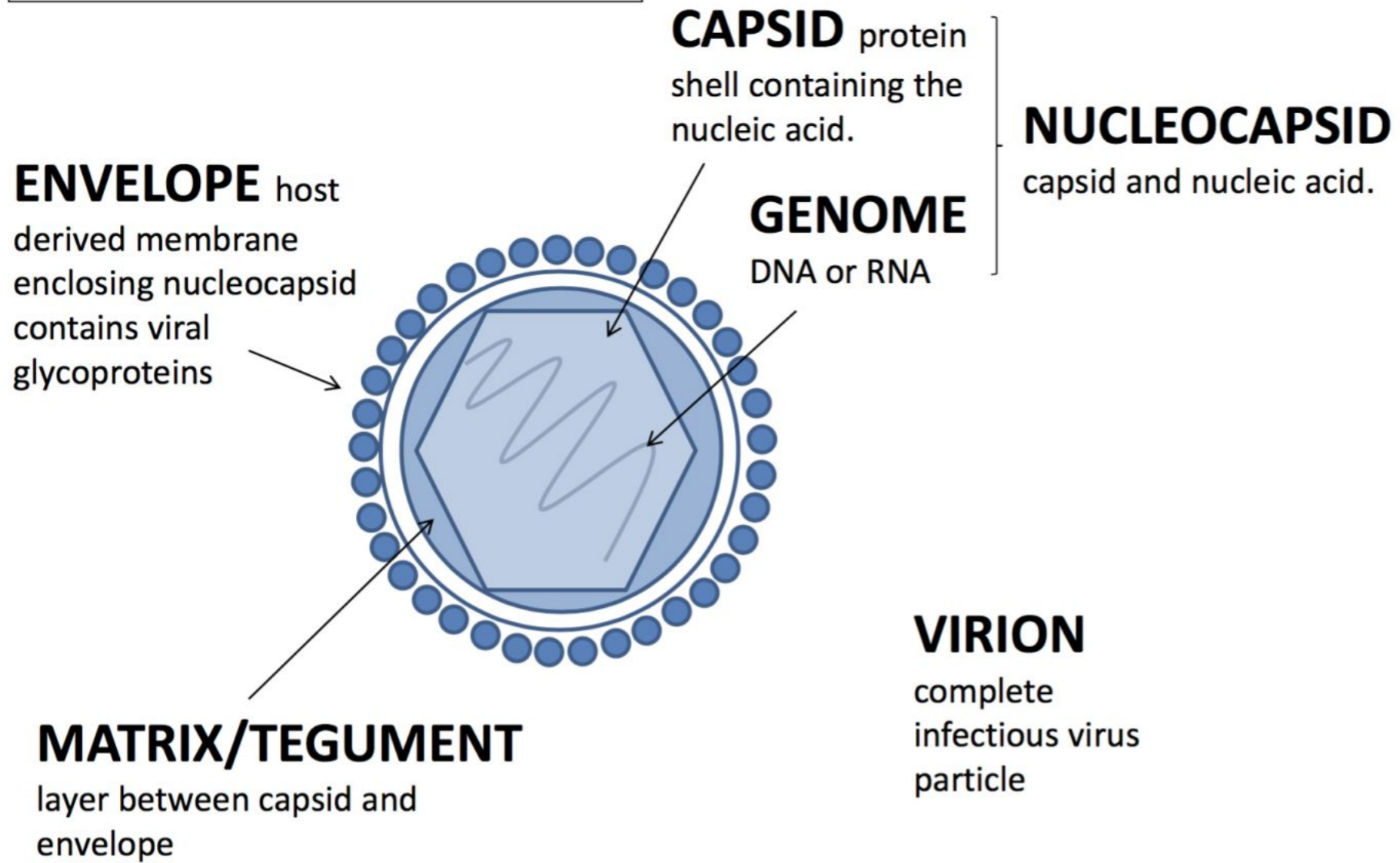


# AB toxins



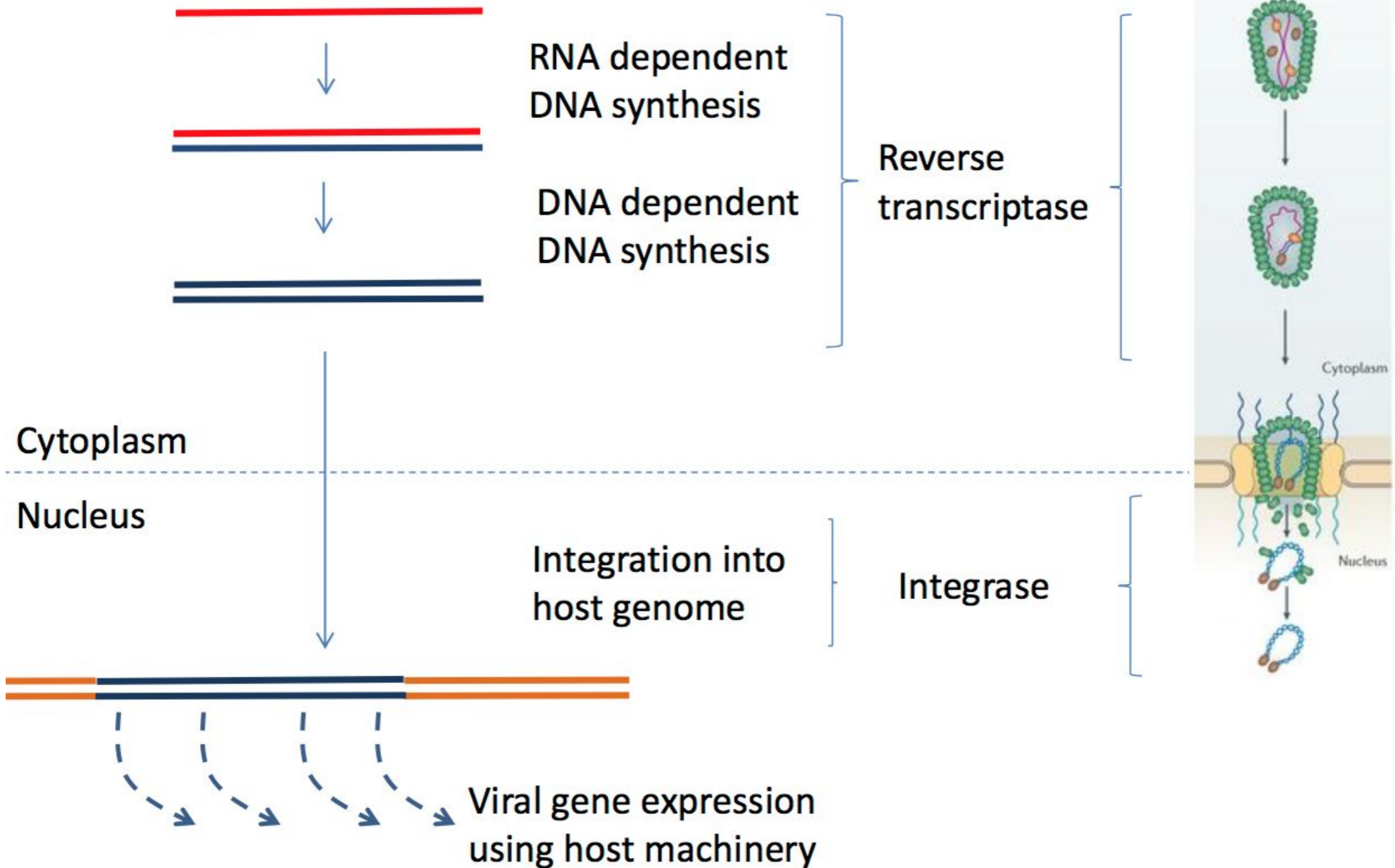
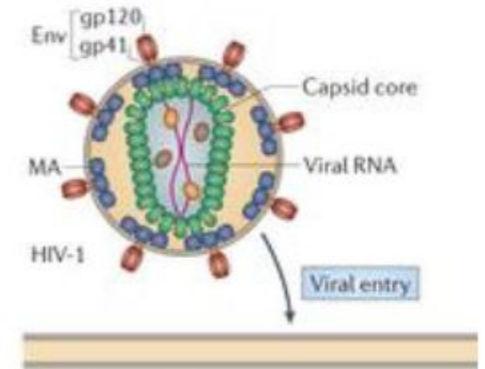
# Viruses

Virus structure: terminology



# Retroviruses - HIV

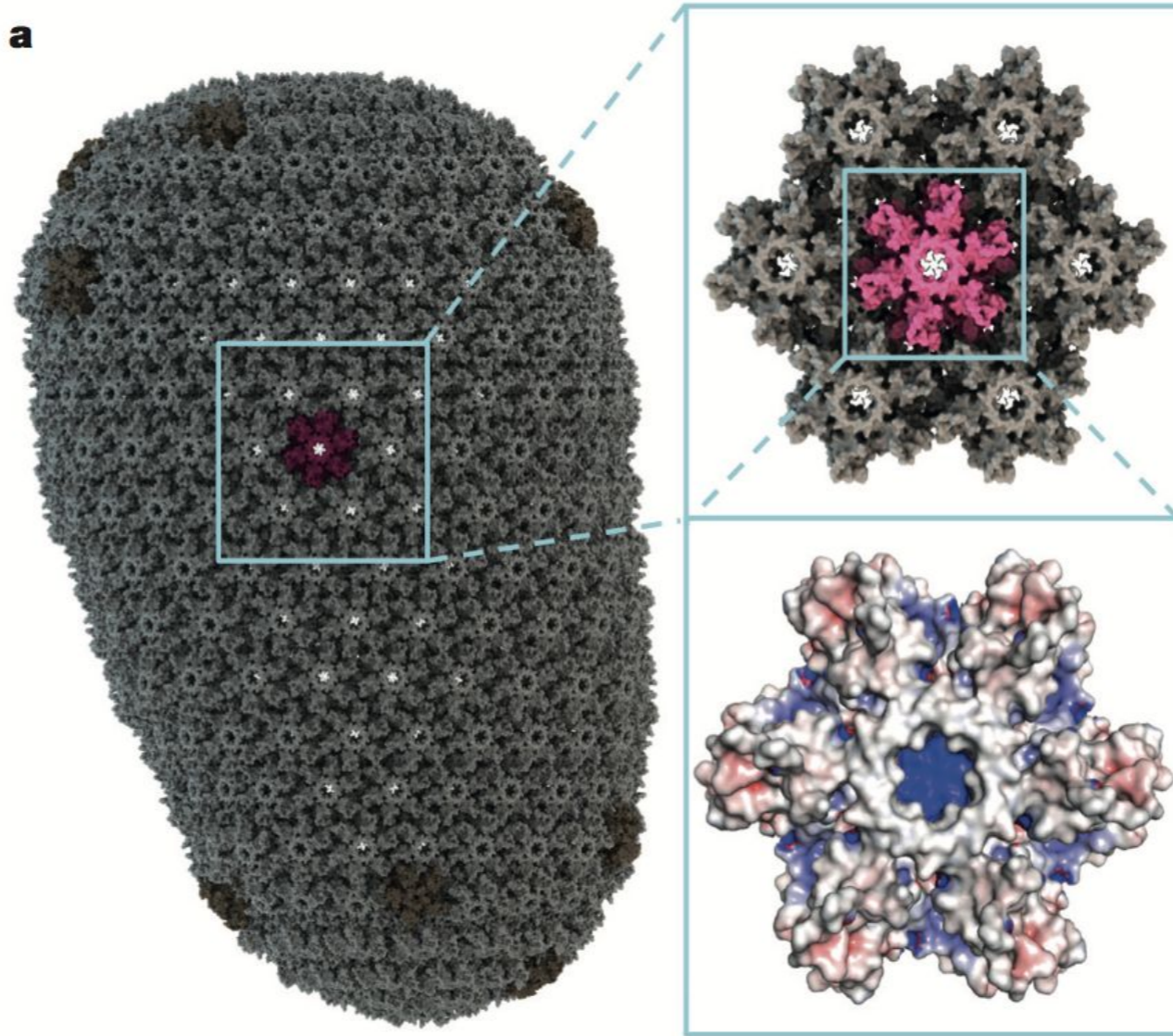
Replication enzymes contained in capsid

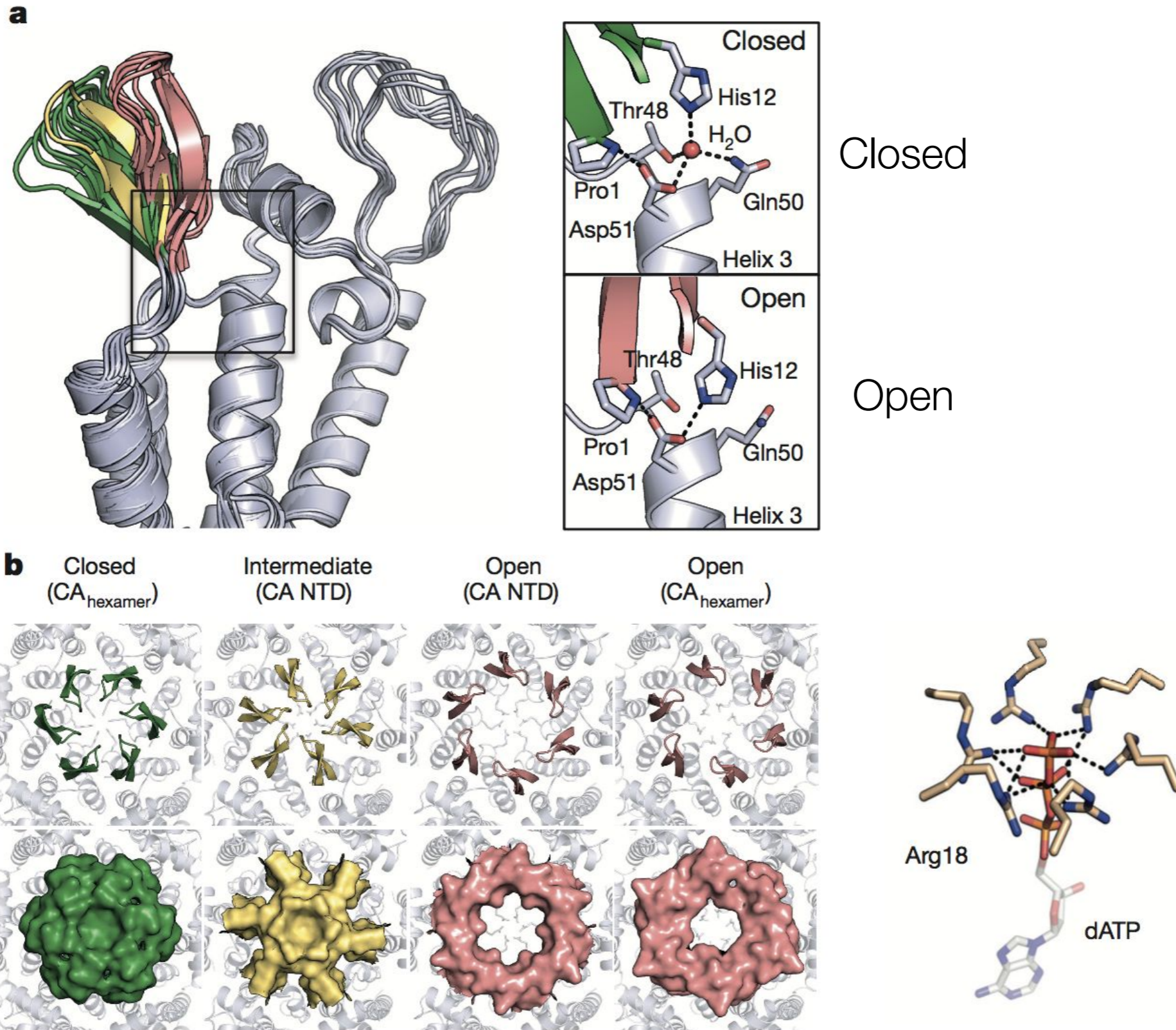




# HIV capsid

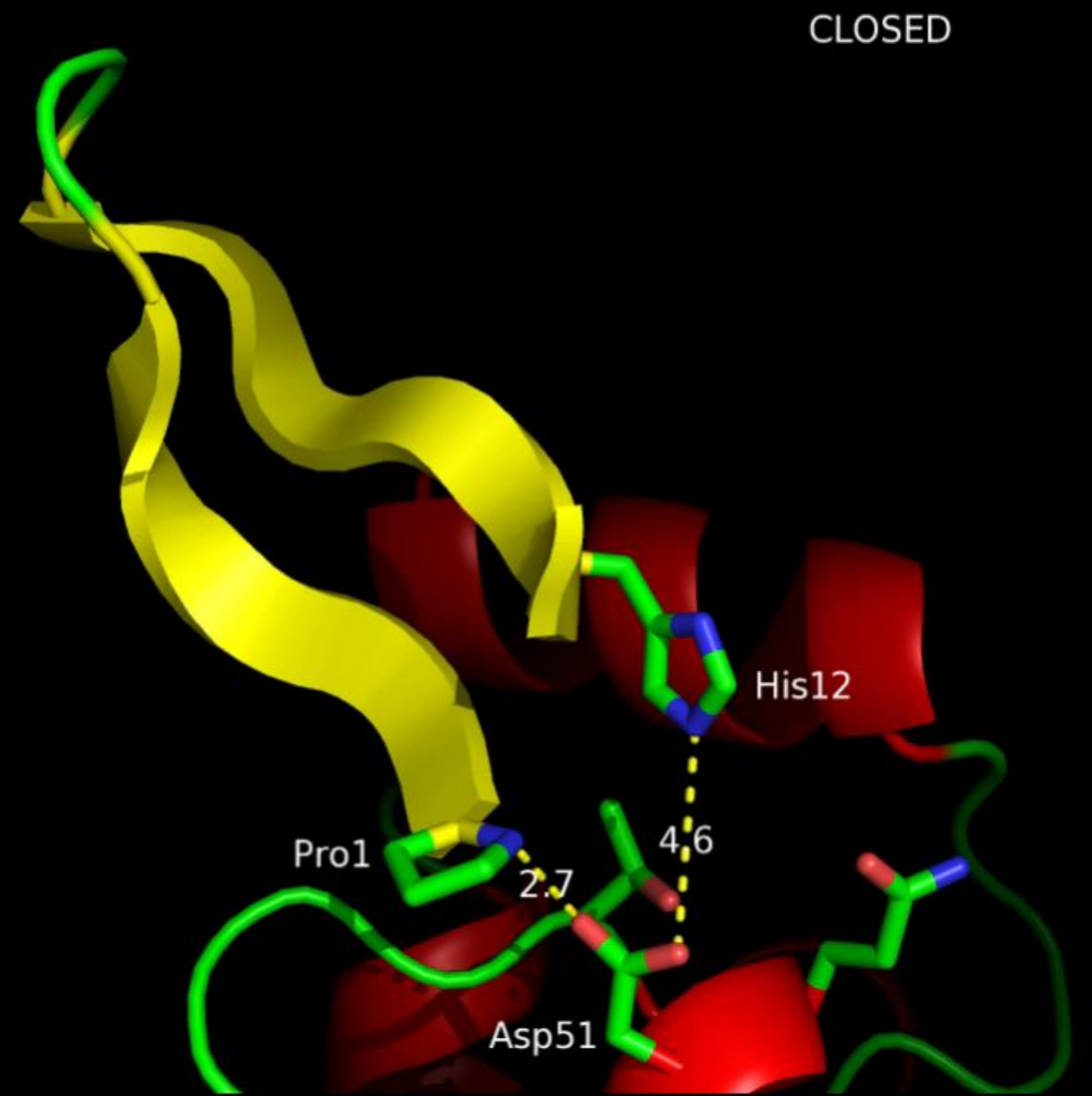
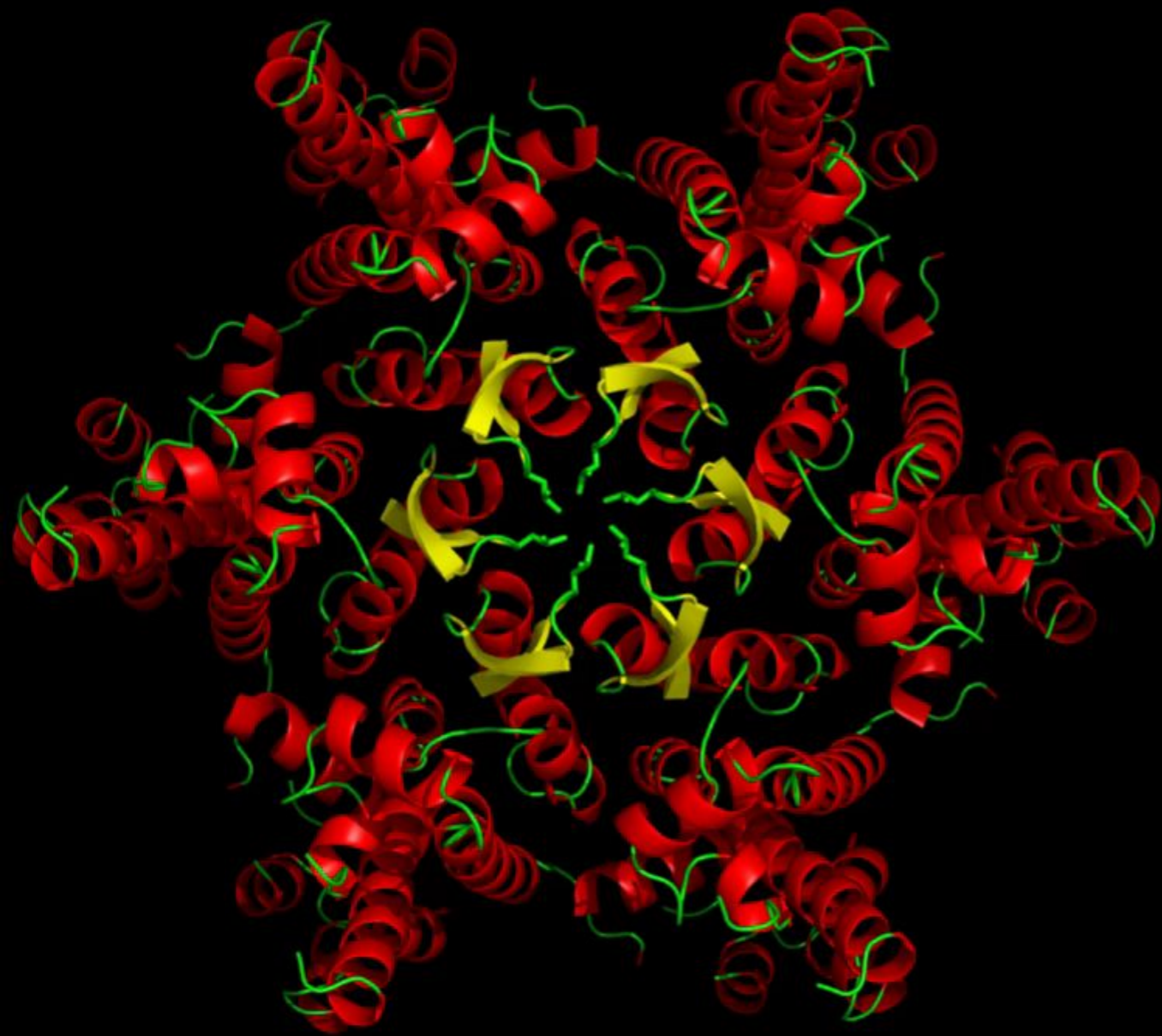
**a**

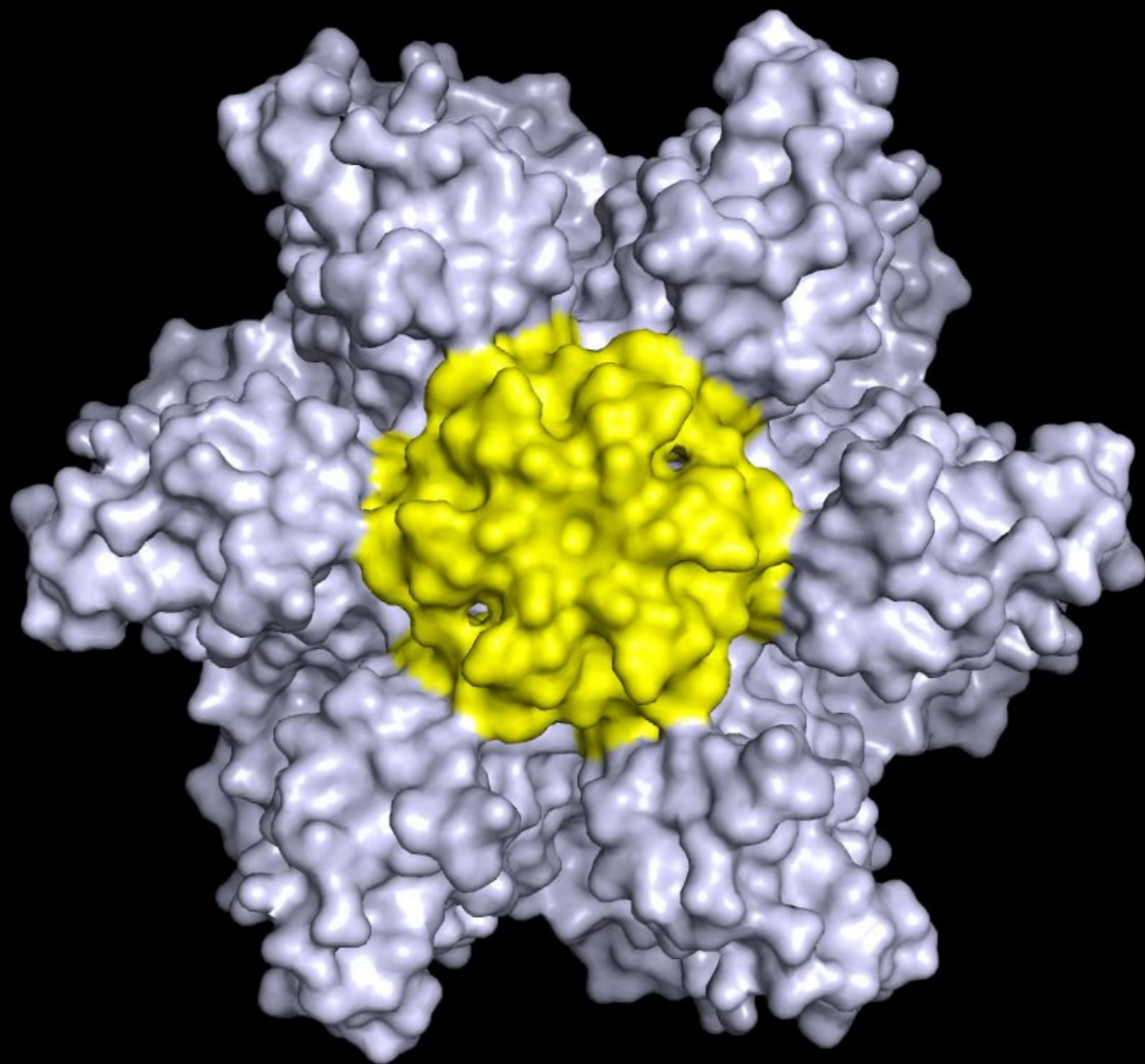




Beta-hairpin can adopt alternate conformations that differ by up to 15 Å.

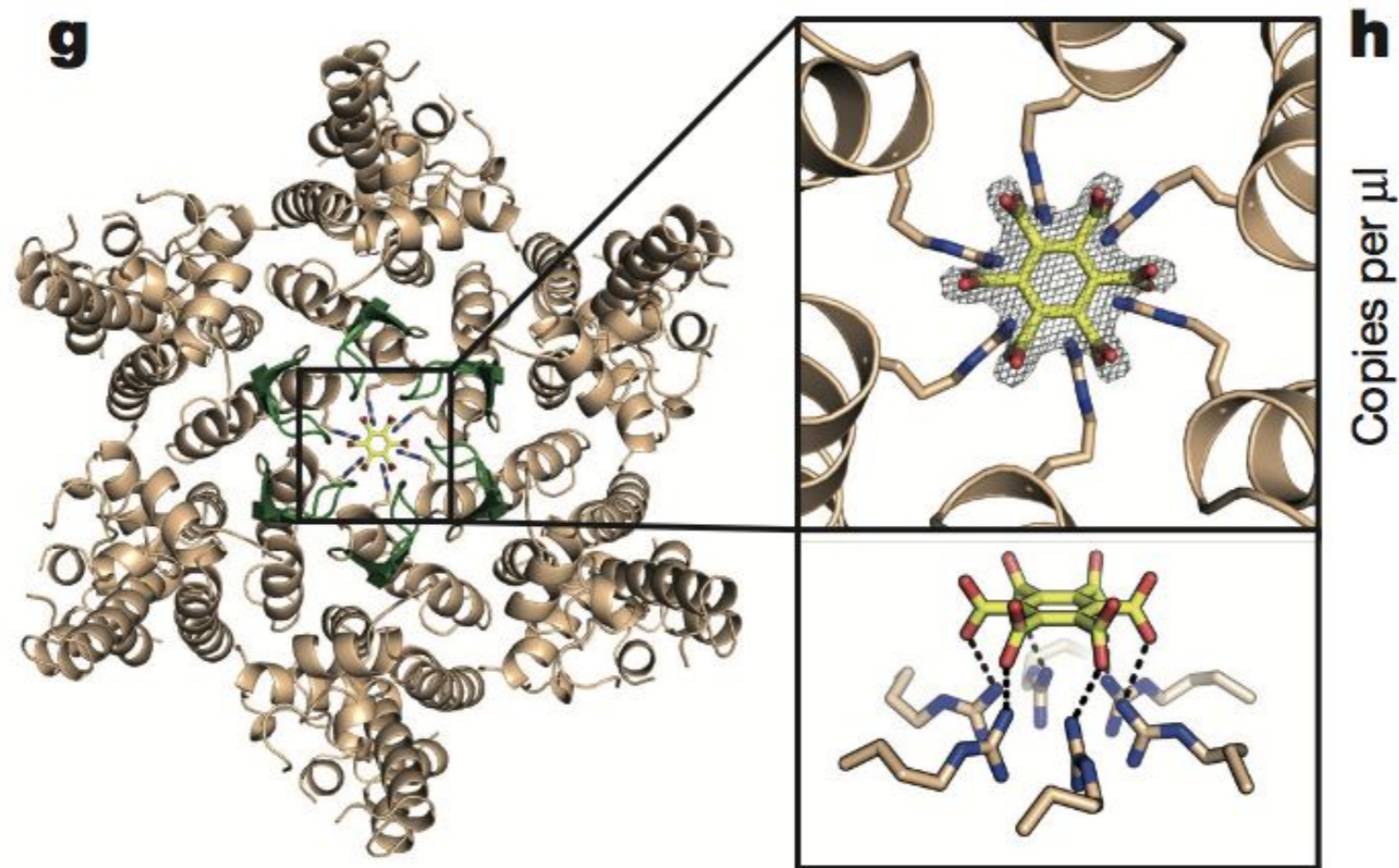








HIV-1 reverse transcription is inhibited by blockade of the capsid pore



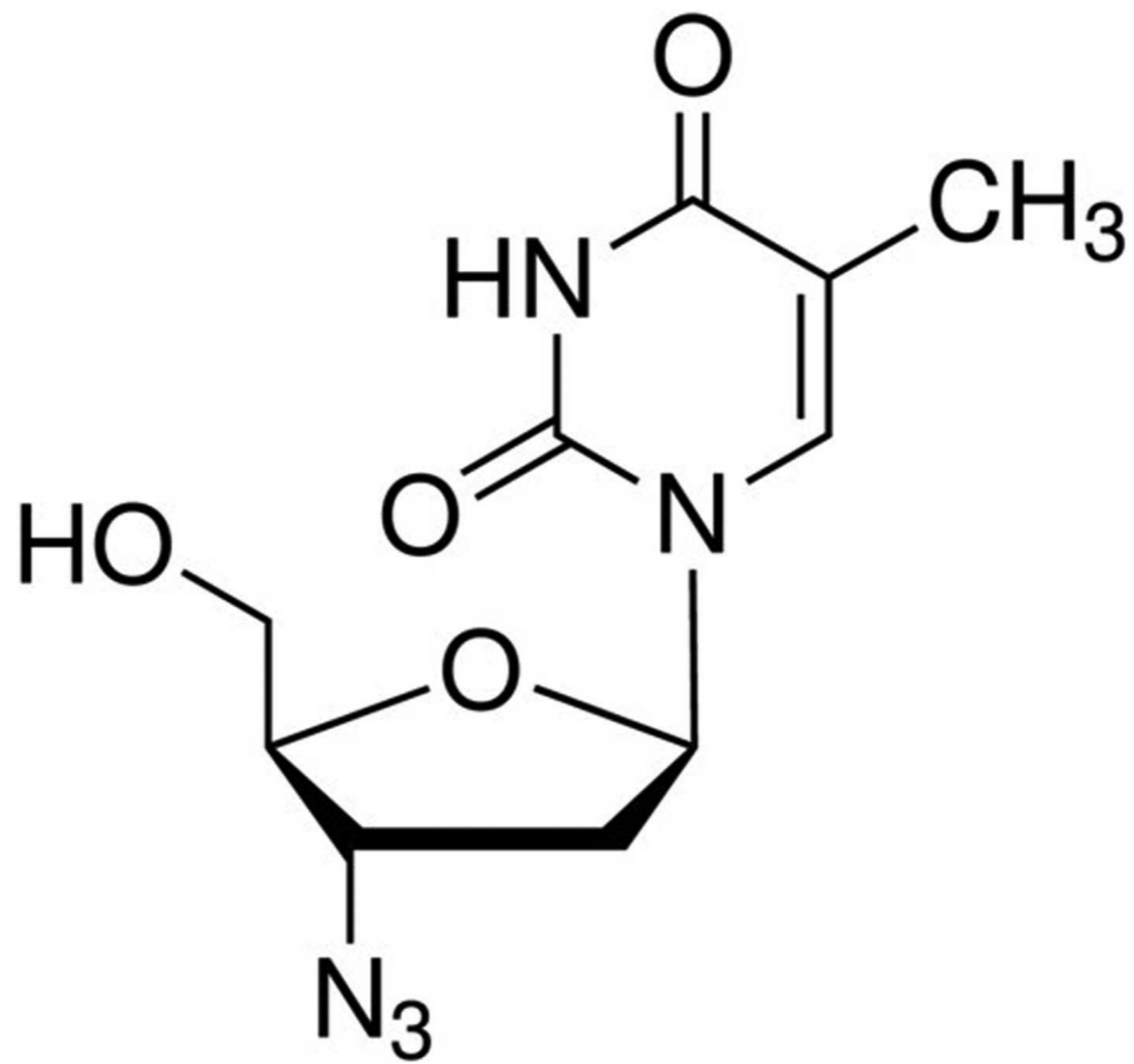
$CA_{\text{hexamer}}$  crystal structure in complex with hexacarboxybenzene, which is co-ordinated by R18

# Current approach to AIDS treatment

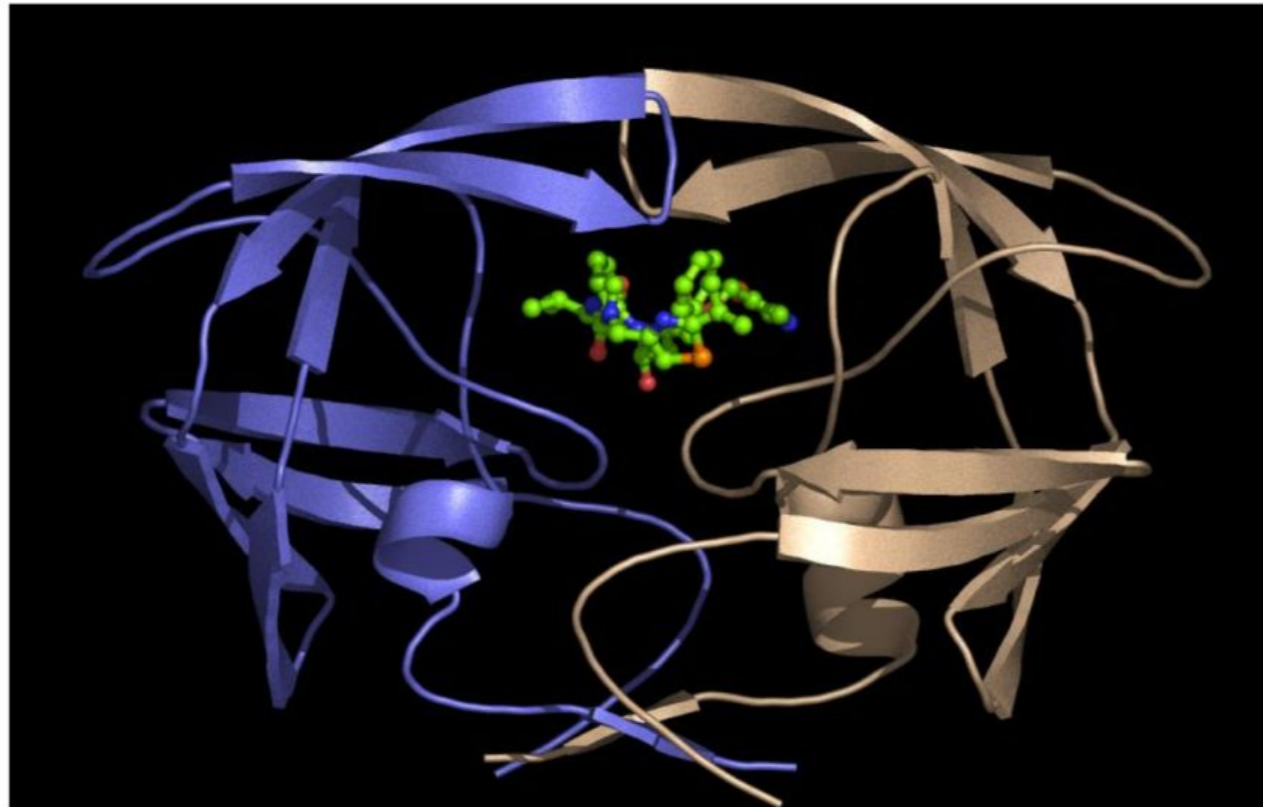
- Nucleoside Reverse Transcriptase Inhibitors
- Protease Inhibitors
- Fusion Inhibitors
- Highly Active Antiretroviral Therapy
- Non-Nucleoside Reverse Transcriptase Inhibitors



AZT



Blocking assembly: protease inhibitors

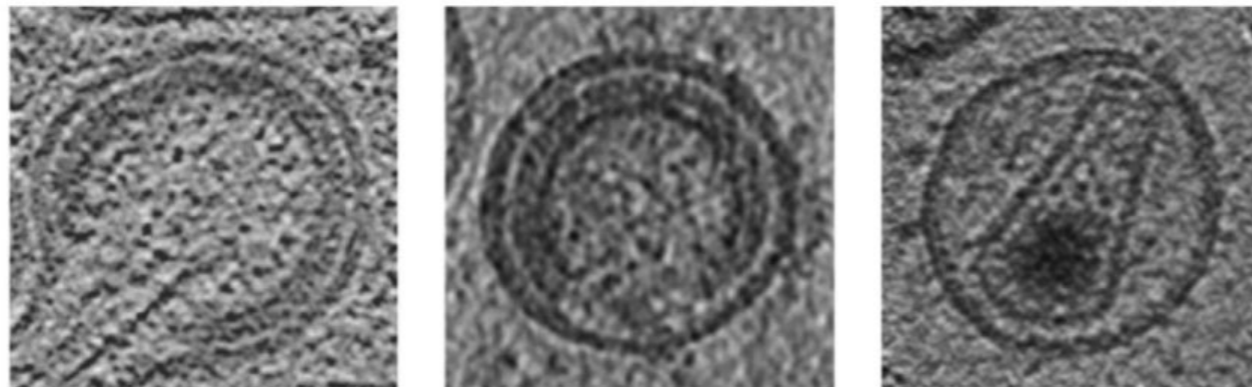


HIV protease

Homo-dimeric  
aspartyl protease

Encoded by pol gene

Peptide mimics of  
cleavage sites are  
inhibitors



Cleaves polyprotein to  
produce capsid  
components