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1 **Similarities and differences between colicin and filamentous phage uptake by bacterial**  
2 **cells.**

3

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5

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15

16 **Abstract**

17 Gram-negative bacteria have evolved a complex envelope to adapt and survive in a broad range  
18 of ecological niches. This physical barrier is the first line of defense against noxious compounds  
19 and viral particles called bacteriophages. Colicins are a family of bactericidal proteins produced  
20 by and toxic to *Escherichia coli* and closely related bacteria. Filamentous phages have a  
21 complex structure, composed of at least five capsid proteins assembled in a long thread-shaped  
22 particle that protect the viral DNA. Despite their difference in size and complexity, group A  
23 colicins and filamentous phages both parasitize multiprotein complexes of their sensitive host  
24 for entry. They first bind to a receptor located at the surface of the target bacteria before  
25 specifically recruiting components of the Tol system to cross the outer membrane and find their

26 way through the periplasm. The Tol system is thought to use the proton-motive force of the  
27 inner membrane to maintain outer membrane integrity during the life cycle of the cell. This  
28 review will describe the sequential docking mechanisms of group A colicins and filamentous  
29 phages during their uptake by their bacterial host, with a specific focus on the translocation  
30 step, promoted by interactions with the Tol system.

31

32

### 33 **Introduction**

34 The cell envelope of Gram-negative bacteria, such as *Escherichia coli*, is characterized  
35 by the presence of two membranes, the inner (IM) and outer (OM) membranes, separated by  
36 the periplasm and a thin layer of peptidoglycan (PG). This envelope is a formidable barrier  
37 against a myriad of harmful compounds, while simultaneously allowing the entry of nutrients  
38 necessary for cell survival. However, this barrier, like the "Maginot Line" in France during the  
39 Second World War, is not completely impenetrable and exogenous particles, including some  
40 toxins and viruses, can pierce it.

41 Colicins are plasmid-encoded toxins (40 to 80 kDa), produced by *E. coli* under  
42 conditions of stress, that allow the efficient killing of related bacteria competing for space and  
43 resources. The toxicity of colicins relies on various modes of action, including IM  
44 depolarization, the inhibition of PG synthesis, and the degradation of DNA or RNA of the target  
45 cell. Colicins can be classified into two groups, A and B, depending on whether they use the  
46 Tol or Ton protein complexes to cross the OM and translocate through the periplasm of their  
47 host (for a review, see 1)

48 Filamentous bacteriophages or *Inoviridae* are elongated viruses (~7 by ~800-2000 nm)  
49 that must pierce the whole envelope to deliver their nucleic acid into the cytoplasm of the target  
50 cell to complete their life cycle. The virion particle consists of an assembly of the major coat

51 protein pVIII, capped with the adsorption protein pIII and minor coat proteins (pVI, pVII and  
52 pIX), encapsulating the circular single-stranded viral DNA. Among inoviruses, Ff coliphages  
53 have garnered particular scientific attention because of their extensive application in genetic  
54 engineering and phage display technology (reviewed in 2), along with the vibriophage CTX,  
55 which converts *Vibrio cholerae* to pathogenicity (3).

56 While colicins and phages are very different particles, their mode of uptake across the  
57 bacterial envelope shares both structural and functional similarities. They both use a multistep  
58 process that relies on the parasitism of structures produced by sensitive bacteria. Group A  
59 colicins and filamentous phages are first specifically recruited via an interaction with a bacterial  
60 surface receptor (reception step), followed by crossing of the OM and transport through the  
61 periplasm (translocation step). This step requires interactions with one or several proteins of  
62 the Tol system, a conserved macromolecular motor of the cell (4). This review describes the  
63 similarities and differences observed between the group A colicin and filamentous phage  
64 models of adsorption and translocation into their host and the role of cellular energy in these  
65 processes.

66

### 67 **Colicins and the Filamentous Phage Adsorption Protein pIII Show Similar Structural** 68 **Organization.**

69 As for many toxins, colicins are organized into three structural domains that perform  
70 specific functions (**Fig. 1A**); the central domain (receptor domain, R) binds a specific bacterial  
71 OM protein, the N-terminal domain (translocation, T domain) is required for colicin  
72 translocation across the OM and interacts with the Tol system, and the C-terminal domain  
73 encodes the toxic activity (5-10). The crystallographic structures of some colicins have  
74 highlighted two important features: long antiparallel coiled-coil  $\alpha$ -helices within the R-domain  
75 that separate this domain from the T and C-domains and a disordered region within the T

76 domain (11-14). Importantly, nuclease colicins are produced in a complex with an immunity  
77 protein, preventing their lethal action in the producing cell (**Fig. 1B**).

78 Filamentous phage infection is driven by three to five copies of the minor coat protein  
79 pIII (also called G3P), located at the tip of the particle (2, 15). As for colicins, phage pIII protein  
80 is organized into three functional domains, separated by two flexible regions (16, 17) (**Fig. 1A**).  
81 The central (N2) domain is responsible for adsorption to a pilus, whereas the N-terminal (N1)  
82 domain interacts with the Tol system. The pIII C-terminal domain anchors pIII to the phage  
83 particle and is required for DNA injection into the host. The structure of pIII-N1 and pIII-N1-  
84 N2 has been solved for the Ff and CTX phages by crystallography and NMR studies (**Fig. 1B**)  
85 (16, 18-22)

86

### 87 **Binding to the Primary Receptor and Crossing of the OM.**

88 The initial cellular reception of most group A colicins requires a high affinity OM  
89 protein receptor, such as BtuB for colicins A and E1 to E9 (**Table1**) (23-25). The structure of  
90 the colicin E3 and E2 R domain (135 residues) bound to BtuB has been solved (26, 27). In the  
91 current model of colicin uptake, the elongated helical coiled-coil R-domain binds BtuB with  
92 the T and C domains extending at a 45° angle out over the OM. Then, the intrinsically  
93 disordered region of the T-domain, adjacent to the OM, searches for a more abundant second  
94 receptor (called “the translocator”), in this case OmpF, and crosses the OM through one of the  
95 OmpF pores, which are wide enough to accommodate unfolded peptides. This model is based  
96 on numerous experiments, such as occlusion of the OmpF channel in planar lipid bilayers by  
97 colicin T domains and isolation of an intact complex of colicin with BtuB and OmpF, confirmed  
98 by crystallization of colicin T-domain peptides within the OmpF pore (28-32). The resulting  
99 Colicin-BtuB-OmpF complex is called the “colicin OM translocon” (**Fig. 2A**).

100 Filamentous phages have been reported to use host-specific Type IV pili or fimbriae  
101 that extend from the cell surface as extracellular receptors (**Table1**). Adsorption of the phage  
102 to the pilus is dependent on pIII-N2, the central domain of the capsid protein pIII (17, 33). For  
103 Ff phages, N2 binding to the *E. coli* F pilus leads to conformational changes in pIII that unmask  
104 the TolA binding site on N1, allowing phage infection to proceed (34) (**Fig. 2B**). How these  
105 phages cross the OM to reach the periplasmic space is unknown, but they may follow pilus  
106 retraction through the pilus secretin that spans the OM (35-37). Interestingly, the reception step  
107 is not strictly required for phage infection, as phages can bypass the pilus to infect cells at low  
108 frequencies (17, 38, 39). In the absence of the F pilus receptor, the addition of CaCl<sub>2</sub> to the  
109 medium has been reported to enhance phage infection, possibly by neutralizing negative  
110 charges at the cell surface (38).

111 After the binding step, which allows specific targeting of the bacterial host, both group  
112 A colicins and filamentous phages parasitize a trans-envelope protein complex, the Tol system,  
113 to cross the periplasm. The Tol-system components required for this step can vary depending  
114 on the group A colicin or phage involved (**Table1**).

115

### 116 **The Tol Macrocomplex: A Conserved Molecular Motor of the Bacterial Envelope**

117 The Tol system is highly conserved among Gram-negative bacteria (40). It consists of  
118 five proteins, TolQ, TolR, TolA, TolB, and Pal, which form a complex in the cell envelope.  
119 TolQRA are IM proteins that interact via their trans-membrane (TM) segments to form an IM  
120 complex (41-44). TolQ is a polytopic protein with three TM segments, whereas TolA and TolR  
121 are bitopic proteins with a large periplasmic domain (43, 45-48). TolB is a periplasmic protein  
122 composed of two subdomains, called D1 and D2, in which D2 forms a six-bladed  $\beta$ -propeller  
123 (49, 50). Finally, Pal is a lipoprotein tethered to the OM via its N-terminal acylated residue, and  
124 its C-terminal domain is free to interact with the peptidoglycan (PG) layer and TolB (51-55).

125 Thus, TolB-Pal forms the OM complex of the Tol system and TolB competes with the PG to  
126 bind Pal (56). The two Tol subcomplexes are transiently connected via the C-terminal domain  
127 of TolA (called TolAIII), which interacts with both TolB-D1 and Pal (57-60). The TolQ-TolR  
128 complex uses the proton motive force (PMF) to induce conformational changes in the  
129 periplasmic domain of TolA, promoting the TolA-Pal interaction (4, 41, 61). Thus, the Tol  
130 system works as a molecular motor, using the PMF to form a link between PG and the inner  
131 and outer membranes.

132 The Tol system has been reported to be essential in many bacterial species, whereas it  
133 is dispensable in the *E. coli* K12 strain (62-66). In *E. coli*, deletion of any of the *tol* genes causes  
134 a pleiotropic phenotype. Indeed, *tol* mutants are highly sensitive to detergents and some  
135 antibiotics, they release periplasmic proteins into the extracellular medium and they form  
136 numerous OM vesicles (67-71). These phenotypes all suggest a potential role of the Tol system  
137 in the maintenance of OM integrity. This role can be partially explained by the involvement of  
138 the Tol complex in OM lipid homeostasis (72, 73). Finally, the Tol system has been described  
139 to be involved in the late stage of the cell-division process, more precisely in OM invagination  
140 during cell division (62, 74).

141

#### 142 **The Tol System Serves as a Versatile Import Machinery for Parasites and Toxins.**

143 Various *tol* mutants have been reported to show resistance both to group A colicins and  
144 filamentous phages. Indeed, direct or indirect interactions of these particles with components  
145 of the Tol complex have been described. When they reach the periplasmic space, most group  
146 A colicins first recruit the  $\beta$ -propeller domain of TolB through their N-terminal T domain (50,  
147 58, 75-77). Conversely, TolB is not required for phage uptake (39, 78). The affinity of ColE9  
148 to TolB has been shown to be strong enough to competitively displace TolB from Pal (77, 79).  
149 Structural data has shown that, in the presence of Pal, the N-terminal 12 residues of TolB,

150 encompassing the TolA binding domain, are ordered and sequestered to the TolB surface,  
151 whereas these residues are disordered and accessible to TolA binding in the presence of the  
152 ColE9 T-domain (79, 80). Thus, in the presence of ColE9, TolB binds to TolAIII and the toxin  
153 can continue its translocation. Although this model can be applied to the other nuclease colicins,  
154 some results suggest that the mechanism for the recruitment of TolB by the pore-forming  
155 colicin A may be different. As ColA is unable to competitively displace TolB in the TolB-Pal  
156 complex, the model suggests that the toxin binds to free periplasmic TolB and then to TolAIII,  
157 for which it has a higher affinity (81) (**Fig. 2A**).

158 All group A colicins and filamentous bacteriophages studied to date require TolA for  
159 their transit into the periplasm (**Table 1**). Direct interaction between the TolAIII domain and  
160 the colicin A, E1, N, and K T-domain has been shown by numerous *in vivo* and *in vitro*  
161 experiments (82-85). Similarly, the pIII protein at the tip of the phage particle is responsible for  
162 TolA binding (19, 22, 86, 87). The phage pIII-N1 and pIII-N2 domains have been reported to  
163 bind *E. coli* TolAIII and TolAII domains, respectively (88). No direct interaction has yet been  
164 observed between TolA and enzymatic colicins, even though *tolA* mutants are tolerant to these  
165 toxins.

166 Several studies indicate that TolA has diverse binding sites, which enable multiple  
167 interactions with other partners. First, point mutations or deletions in *tolA* differentially affect  
168 colicin import; some remain active, whereas others lose their ability to kill mutated strains (59,  
169 89). Second, structural data have shown that the colicin A T-domain interacts with the convex  
170 site of TolAIII, whereas the phage Ff and IF1 pIII-N1 domain and colicin N T-domain bind to  
171 the concave side of TolAIII (18, 21, 90-93) (**Fig. 1B**). In contrast to coliphage Ff, the  
172 vibriophage CTX pIII-N1 domain binds to the convex site of *V. cholerae* TolAIII (22).

173 The role of the TolQ and TolR proteins in both phage and colicin uptake is less clear.  
174 Although TolQ and TolR are necessary but not essential for phage infection (39, 94), both are



175 absolutely required for the translocation of group A colicins (excepted ColE1). However, only  
176 a few studies have reported a direct interaction between colicins and either TolQ or TolR. The  
177 ColA and ColE3 T-domain has been shown to interact with TolR by cross-linking experiments  
178 (95) and the interaction between the ColK T-domain with TolQ or TolR has been revealed by  
179 coimmunoprecipitation (85). The final steps concerning the toxic effect of colicins and phage  
180 DNA injection are not discussed here but are briefly summarized in **Figure 2**.

181

### 182 **Importance of Protein Structural Flexibility in Uptake Pathways.**

183 An interesting aspect of the current models of colicin uptake is that their T-domains are  
184 disordered and that they fold into an ordered structure upon binding to a Tol protein. This  
185 disorder-to-order transition could explain the ability of colicins to bind several target partners  
186 and progress through the periplasm.

187 Another important aspect in both colicin and phage translocation is the large-scale  
188 structural modification of the molecules during the process. First, studies have shown that  
189 colicins A and E2 remain bound to their receptor and the Tol machinery, whereas their C and  
190 T domains are translocated (96, 97). Second, *in vivo* and *in vitro* experiments have shown that  
191 colicin A unfolds during its import into the cells (98, 99). This unfolding is triggered either by  
192 binding to the first ColE1 receptor, which is then propagated to the distal ends of the T and C  
193 domains of the toxin (26, 100), or it occurs after recruitment of the second receptor and  
194 formation of the “colicin OM translocon” for ColE9 (101). Similarly, the extensive structural  
195 modifications of coliphage pIII protein during initial binding to the F-pilus, followed by  
196 interaction with TolA in the periplasm, have been proposed to lead to the insertion of pIII-C  
197 into the IM of the host and the formation of a multimeric channel that allows phage DNA  
198 injection into the bacterial cytoplasm, concomitant with disassembly of the capsid by diffusion  
199 of the major coat protein pVIII in the membrane (102, 103).

200

## 201 **Energetics of Colicin and Filamentous Phage Uptake.**

202         Despite the large number of publications on colicin and phage import, only a few studies  
203 have focused on the energetic aspect of this process. As discussed above, the Tol system use  
204 the IM PMF, by means of the TolQR motor, to power mechanisms involved in the maintenance  
205 of OM integrity and cell constriction. However, initial studies suggested that the Tol-dependent  
206 import of two pore-forming colicins, ColA and ColE1, is energy-independent (104, 105). These  
207 results were later confirmed by point-mutations introduced into the TolQR motor that affect the  
208 physiological function of the Tol system without preventing the killing action of pore-forming  
209 colicins. These mutations were called “discriminative mutations” (106, 107). Energy  
210 requirements for the import of nuclease colicins appear to be different. It is clear that release of  
211 the immunity protein from nuclease colicins in the external medium requires functional import  
212 machinery (108, 109). Intriguingly, the release of the ColE9 immunity protein was shown to be  
213 energy dependent (109), whereas strains harboring discriminative TolQR mutations remain  
214 susceptible to ColE2 (107). Based on these divergent results, the question of the energy  
215 requirement for the translocation of group A colicins is still open. For coliphages, experiments  
216 using arsenate or protonophores has led to the hypothesis that phage uptake may be dependent  
217 on both ATP and PMF, possibly for pilus retraction and phage particle transit through the  
218 periplasm, respectively (110, 111).

219

220         In summary, it is fascinating to observe that small toxins, such as colicins, and complex  
221 viral structures, such as filamentous phages, are shaped so as to use similar molecular  
222 mechanisms to pierce the envelope of their target cell. These include a sequential docking  
223 mechanism, the strict requirement for the hub protein TolA, and large-scale structural  
224 modifications of the proteins during their transit. Although some key steps in these processes

225 have been well characterized, numerous questions are still unanswered concerning the  
226 dynamics, sequence of events, and role of host-cell energy in the uptake of these  
227 nanostructures.

228

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712

713 **Table1.** Host proteins required for reception and translocation of filamentous phages and group A colicins.

714

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715	Name	Receptor	Translocator	Uptake Proteins	Cytotoxicity/Host	Ref.
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717

718 **Group A colicins**

719

720	ColA	BtuB	OmpF	TolA, B, Q, R	Pore-forming	
721	ColE2, E7, E8, E9	BtuB	OmpF	TolA, B, Q, R	Dnase	
722	ColE3, E4, E6	BtuB	OmpF	TolA, B, Q, R	rRNase	
723	ColE1	BtuB	TolC	TolA, Q	Pore-forming	
724	ColN	LPS	OmpF	TolA, Q, R	Pore-forming	(112)
725	ColE5	BtuB	OmpF	TolA, B, Q, R	tRNase	
726	ColK	Tsx	OmpF/OmpA	TolA, B, Q, R	Pore-forming	(113)
727	ColU	OmpA	OmpF	TolA, B, Q, R	Pore-forming	(114)

728

729 **Filamentous phages**

730	Ff (Fd, f1, M13)	F-pilus		TolA, Q, R	<i>E. coli</i>	
731	IKE	N-pilus		TolA, Q, R	<i>E. coli</i>	(115)
732	IF1	I-pilus		n.d.	<i>E. coli</i>	
733	CTX	TCP pilus		TolA, Q, R	<i>V. cholerae</i>	
734	VGJ	MshA pilus		n.d.	<i>V. cholerae</i>	(116)
735	Pf1	type IV PAK pilus		n.d.	<i>P. aeruginosa</i>	(117)
736	Pf3	RP4 pilus		n.d.	<i>P. aeruginosa</i>	(117)

737

738 Only references that do not appear in the text are cited in this table. n.d.: not determined.

739



740 **Figure legends**

741 **Figure 1. Colicin and phage minor coat protein organization and crystal structures. (A)**

742 Schematic representation highlighting the similar general organization of colicin and phage pIII  
743 proteins for Translocation (T or N1 domains), Reception (R or N2 domains), and Activity or  
744 Anchoring (A or C domain), respectively. **(B)** Structures of: full length colicin E3 (top left,  
745 PDB: 1JCH) bound to its immunity protein (in green); full length colicin N (top right, PDB:  
746 1A87); M13 phage protein pIII N1-N2 domains (bottom left, PDB: 1G3P); and superposition  
747 (bottom right) of *E. coli* TolAIII domain (grey) interacting with the colicin A T-domain on its  
748 convex side (co-crystal PDB: 3QDR) and *E. coli* TolAIII domain (grey) interacting with G3P-  
749 N1 on its concave side (co-crystal PDB: 1TOL). The color code used for each protein domain  
750 is the same for panels A and B.

751

752

753 **Figure 2. Models of group A colicin (A) and Tol-dependent filamentous phage import (B)**

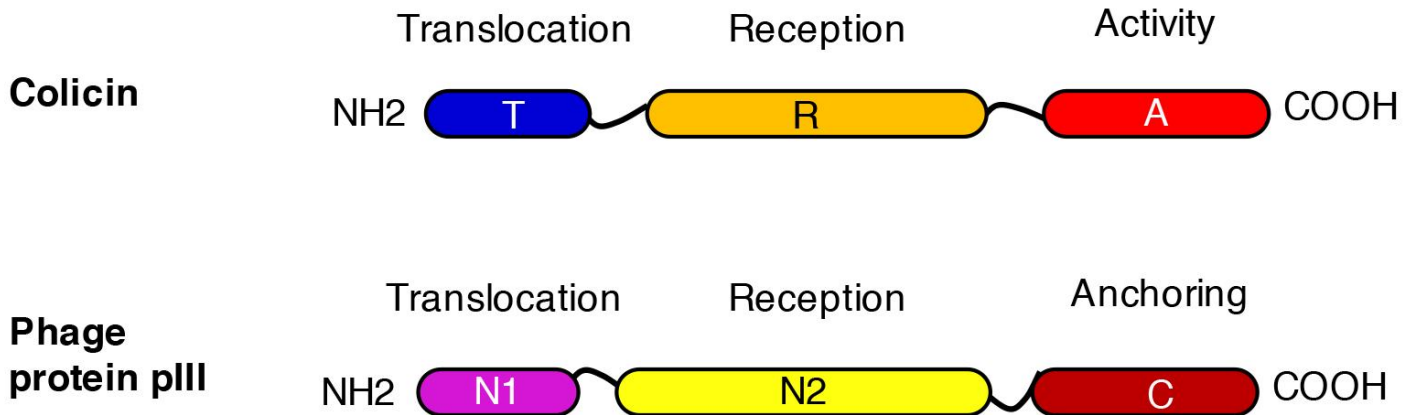
754 **into sensitive *E. coli* cells. (A)** In stage 1, colicin binds to the OM receptor by its central domain  
755 (26, 27). In stage 2(a), the disordered N-terminal segment of the T-domain translocates through  
756 the OM  $\beta$ -barrel and interacts with a free periplasmic TolB or dissociates TolB from Pal (28-  
757 31, 77, 79, 81). In stage 2(b), the N-terminal segment interacts with other Tol proteins (82-85,  
758 95). At this stage, the immunity protein of nuclease colicins is released (108, 109). Then, the  
759 unfolded C-terminal domain is thought to cross the OM through the interface between OmpF  
760 and the lipid bilayers (118) or directly through the OmpF porin (28). In stage 3, for pore-forming  
761 colicins (i) the C-terminal domain inserts spontaneously into the IM and form voltage-gated  
762 channels that depolarize and kill the target bacteria (for a review see, 119). For nuclease colicins  
763 (ii), the C-terminal domain is cleaved by FtsH (120, 121), an essential ATP-dependent IM  
764 protease, and spontaneously crosses the IM (122) or uses FtsH for its transfer (121). **(B)** In stage

765 1, the phage minor coat protein pIII-N2 domain binds to the tip of a F-pilus protruding from the  
766 cell surface (33). In stage 2, pilus retraction pulls the phage into the cell periplasm, possibly  
767 through the pilus secretin pore. Once there, the phage pIII-N1 domain interacts with the  
768 globular domain of TolA (TolAIII) (86, 87). In *E. coli*, a direct interaction between TolAII and  
769 phage pIII-N2 has been reported (dash arrow) (88). The PMF-dependent TolQR motor may  
770 trigger conformational changes of TolA that brings the phage particle in close contact with the  
771 IM. The phage uncapping process during the uptake stage (3) is speculative. In the model, pIII  
772 oligomerizes to form a channel in the IM of the host through its C-ter domain (pIII-C). Then,  
773 diffusion of the phage pVIII major coat protein in the IM leads to disassembly of the capsid,  
774 releasing the internal pressure of the structure. This force is thought to drive phage DNA  
775 injection through the IM pIII-C channel (102, 103).

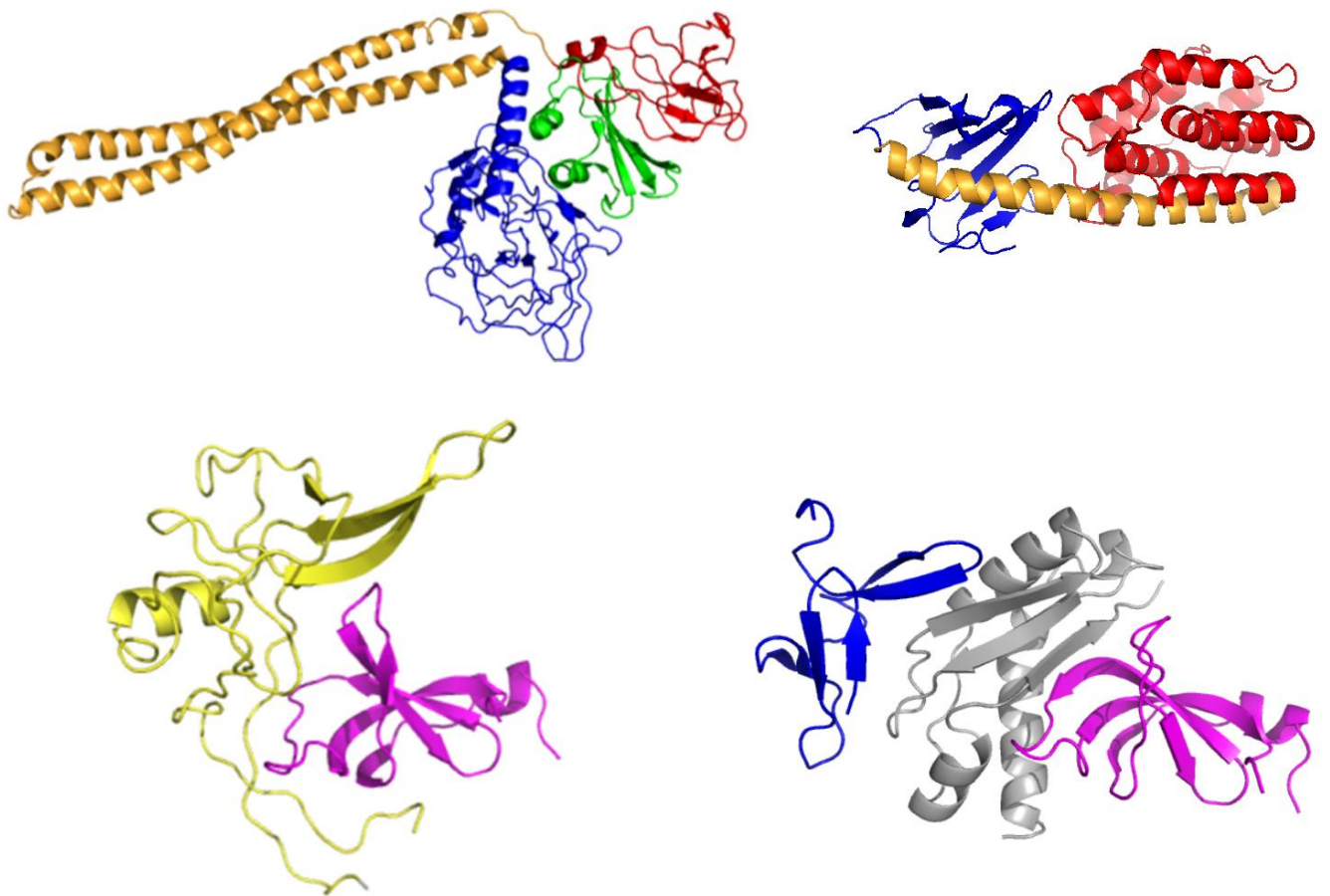
776 OM, outer membrane; IM, inner membrane; PG, peptidoglycan; peri, periplasm; cyto,  
777 cytoplasm; rec, receptor; trans, translocator. The phage is composed of three to five copies of  
778 pIII, but only one copy has been represented, and other minor virion coat proteins have been  
779 omitted for simplicity.

780

**A**



**B**



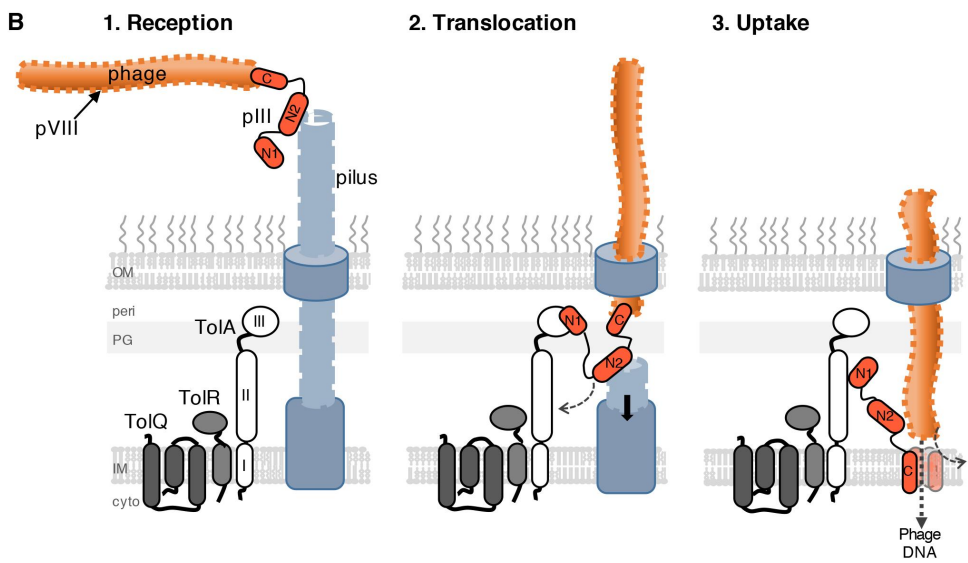
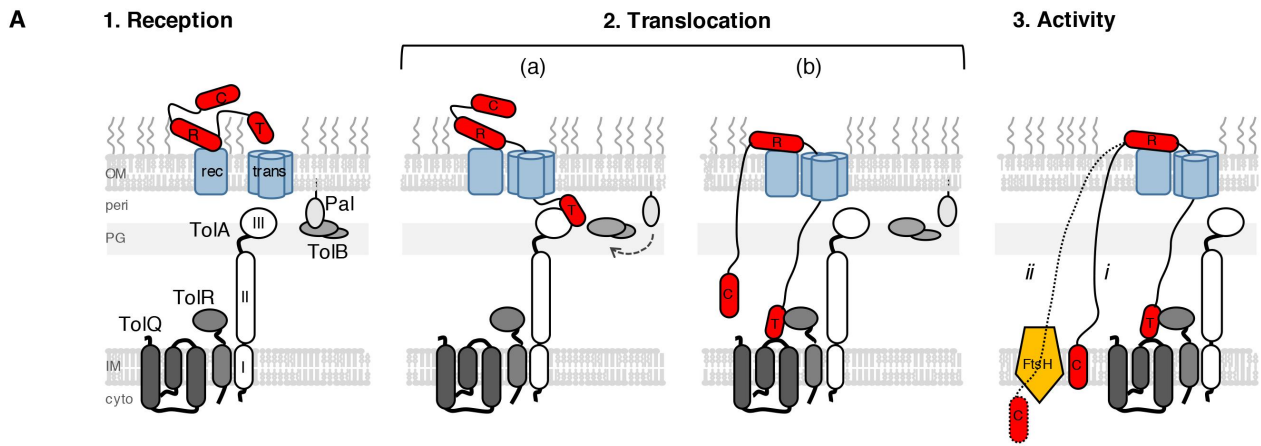


Figure: 2