

Structural lessons on bacterial secretins

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1 STRUCTURAL LESSONS ON BACTERIAL SECRETINS

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13 ABSTRACT

To exchange and communicate with their surroundings, bacteria have evolved multiple active and passive mechanisms for trans-envelope transport. Among the pore-forming complexes found in the outer membrane of Gram-negative bacteria, secretins are distinctive homo-oligomeric channels dedicated to the active translocation of voluminous structures such as folded proteins, assembled fibers, virus particles or DNA. Members of the bacterial secretin family share a common cylindershaped structure with a gated pore-forming part inserted in the outer membrane, and a periplasmic channel connected to the inner membrane components of the corresponding nanomachine. In this

- 21 mini-review, we will present what recently determined 3D structures have told us about the 22 mechanisms of translocation through secretins of large substrates to the bacterial surface or in the
- 22 mechanisms of transloc23 extracellular milieu.
- 23 extrat 24

25 **KEYWORDS**

- 26 bacterial secretin; beta-barrel; outer membrane; cryo-EM; structure-function
- 27

28 HIGHLIGHTS

- 29 Secretins are the OM portal of the T2SS, T3SS, T4P and FPE systems
- 30 Secretins form giant pores tailored to the secretion or assembly of large structures
- 31 Secretins consist of a conserved OM C-module and a variable periplasmic N-module
- 32 The channel formed by the C-module is occluded by a flexible central gate (CG)
- 33 The N-module connect with the IM components of the respective systems

34 1. Introduction

Secretins were discovered in the 80's during the study of Klebsiella oxytoca outer membrane (OM) 35 36 constituents responsible for the export of folded pullulanase to the extracellular milieu [1]. Secretins 37 have been intensively since because of their unique ability to accommodate large folded exoproteins 38 and protein complexes in several bacterial transport nanomachines [2]. Due to their function, secretins 39 were thought for decades to form giant ring-shaped homo-oligomers in the OM [3-8], but their precise 40 atomic organization was deciphered only recently thanks to spectacular advances in cryo-electron 41 microscopy (cryo-EM) [9, 10]. This revealed extraordinary ternary and quaternary networks, 42 highlighting unsuspected structural determinants at the basis of functions hitherto observed but not 43 yet understood. In this mini-review, we will present how cryo-EM structural data shed light on the 44 architecture and function of bacterial secretins, and how structural determinants compartmentalize 45 and animate these giant pores embedded in the bacterial envelope.

46

47 **2.** The bacterial secretin family

48 The existence of a bacterial secretin family, first proposed by Genin and colleagues, comprised several 49 OM-associated proteins essential for the transport of macromolecules across bacterial membranes [11]. Bacterial secretins are widely used by bacteria and even by mitochondria [12] in a variety of 50 51 transport systems specialized in the transit of large molecules across the OM. These range from 52 delivery of folded effectors by the Type 2 Secretion System (T2SS) to filament accommodation in the 53 case of the Type 3 Secretion System (T3SS) and Type 4 Pili (T4P) or phage release in the Filamentous 54 Phage Extrusion (FPE) system. Members of this family were historically defined by a highly conserved 55 C-terminal "secretin domain" which appeared to be the OM pore-forming domain [13], and a variety 56 of N-terminal domains, combinations of distinct modular domains connected via short linkers, whose 57 number depends on the transport machine [14]. For example, while the T2SS-secretin N-part is composed of three repeated N3, N2 and N1 Ring-Building Motif (RBM) domains and a N0 TonB-58 59 dependent transduction domain [15] possibly supplemented by a peptidoglycan binding SPOR domain 60 [16], the N-part of the other secretin types (T4P and FPE) are made up of N3 and N0 domains, optionally 61 completed by an internal N1 sub-domain for T3SS or two distal amidase N-terminal "AMIN" domains 62 for T4P [17]. Finally, the T2SS and T3SS secretins possess an additional extreme C-terminal S-domain, 63 the attachment site for a partner protein called pilotin, which assists secretin assembly and targeting to the OM (see [18] for review). 64

65

66 **3. Cryo-EM reveals atomic 3D architecture of secretins**

67 <u>3.1 Overall organization</u>

68 Recent advances in the field of cryo-EM applied to isolated biological samples led to high-resolution 69 3D structures for at least one member of each bacterial secretin type (see Table 1 for an exhaustive 70 list of secretin near-atomic-resolution cryo-EM structures available). These structures – illustrative 71 examples are presented Figure 1 - revealed a bimodular organization with a well-defined OM C-72 module, followed by a periplasmic variable N-module composed of several superposed ring-like 73 structures connected by short linkers. In most secretin structures, the C-module is resolved at a 74 resolution of 3-4 Å, whereas the resolution decreases over the N-module to become poor in the most 75 N-terminal N0 domain. It has been hypothesized that this results from the N0 domain high flexibility 76 in absence of its inner membrane (IM) interacting partner. This is in line with N0 high resolution in the 77 structure of the T2SS PulD secretin of Klebsiella pneumoniae copurified with its IM partner PulC [19]. 78 The C-module found in all secretins has a structural organization into three domains, which could not be predicted from the primary sequence. First, there is a giant double wall β-barrel with a closed central gate (CG) that must open to allow the passage of the translocated structures. In some T2SS secretins, the channel is capped by an additional top gate (TG). Second, we consider the N3 RBM-domain, originally thought to be part of the N-domain, as part of the pore-forming C-module, which is consistent with its structural role and conservation in secretins [20]. Finally, the extreme C-terminal S-

- 84 domain (specific of T2SS and T3SS-secretins) is composed of 2 α -helices that encircled the outer barrel.
- 85

86 <u>3.2. Stoichiometry</u>

87 The exact stoichiometry of secretins has always been and still is a matter of debate. Previous low-88 resolution 3D structures suggested a homo-multimeric organization of 12, 14 or 15 subunits depending 89 on the type [14]. The recently reported high-resolution structures (listed in Table 1) are compatible 90 with these stoichiometries, at least for the C-modules. Either isolated or in complexes, T2SS, T3SS and 91 FPE secretins C-modules present a definite pentadecameric (C15) symmetry, whereas T4P secretins 92 primarily adopt a tetradecameric (C14) symmetry. Interestingly, different stoichiometries have 93 sometimes been reported for a given secretin C-module, e.g., 28 % of the Escherichia coli T2SS GspD 94 are hexadecameric (C16) [10], while 8 % of the Pseudomonas aeruginosa T4P PilQ adopt a C15 95 symmetry [21]. This variability does not appear to be restricted to secretin types, and it could therefore 96 constitute an additional adaptation to accommodate complex secretion/assembly processes.

97 The stoichiometry of N-modules is less well understood since their 3D structures are poorly, 98 or not at all, resolved, probably due to high intrinsic flexibility. This is regrettable because there is a 99 recurrent symmetry conflict for T3SS and T2SS secretins between C-modules and IM partners, which 100 present incompatible 15:24 and 15:12 stoichiometries, respectively [19, 22]. Recently, structures of 101 T3SS complexes including secretins and their IM partners indicate that the stoichiometry mismatch 102 could be resolved by the presence of additional secretin domains. In the case of Salmonella enterica serovar Typhimurium T3SS, the presence of a sixteenth secretin subunit is clearly seen for the N0-N1 103 104 domains, leading to a compatible InvG₁₆:PrgH₂₄ interface with 8 additional PrgH subunits packing 105 around and under the β -sheet to reinforce the interface [22]. The addition of N0-N1 monomers in a 106 pentadecameric secretin was also observed for the Shigella flexneri T3SS secretin MxiD [23, 24]. For 107 T2SS, the interface region between the PuID N0 domain and its IM partner PuIC is also puzzling with 108 an estimation of around 15 PulD-N0 for about 12 PulC-HR, leaving up to three PulD-N0 unoccupied 109 [19]. Independent work showed that the *P. aeruginosa* T2SS secretin XcpD (formerly XcpQ) adopts a 110 dodecameric (C12) conformation when purified without the C-module [25]. As proposed by Hay and 111 colleagues [26], such symmetry mismatch in T2SS secretins might be overcome by the displacement of three XcpD N-modules away from the XcpC IM-partner-interface, thus leading to the formation of 112 113 the metastable C15:N12 arrangement. The overall stoichiometry of a given secretin might therefore 114 fluctuate, especially in its N-terminal part, to be compatible with the stoichiometry of its IM partner. 115 However, additional structural data is needed for a more complete understanding of this conundrum.

116

117 <u>3.3. Charge distributions</u>

While their structures are similar, the internal electrostatic characteristics of bacterial secretins vary significantly from one secretin type to another but also within the same type (figure 2) [27, 28, 30]. The overall amount of charged amino acids is around 20 % depending on the secretin's type, but their distribution on the quaternary structure is not homogeneous along the channel of each secretin. On the structures available to date, it seems that the entry of the internal cavity in the N-terminal side has a relatively positive charge, whereas in the N3 domain it turns to be relatively negative except for FPE

124 secretins. An important charge heterogeneity is found in a disordered loop in the N3-domain of 125 variable size, but neither its structural importance nor its functional role have been determined yet. 126 The variation of charge in the lower cavity could be important to stabilize the assembled filament inside 127 the secretin, or to allow the docking of the folded effector in T2SS, but these hypotheses have never 128 been investigated. Looking at the CG of T3SS secretins, we observe a predominant charge that is 129 opposite to the charge present on the wall of the upper cavity. Such charge distribution could allow 130 stabilization of the central gate once it is open considering that it stays open in this secretin when the 131 needle is assembled. The charge compatibility between the top of the CG and the β -barrel wall is not seen in the T2SS, T4P and FPE secretins where these regions display relatively similar charge 132 133 distributions. In terms of dynamics, the presence of identical charges in these zones would lead to 134 electrostatic repulsion and therefore could allow rapid closing of the CG when the pseudopilus or pilus 135 is disassembled in the T2SS and T4P systems or when the phage particle has been secreted in the FPE system. If we compare the different secretin types, the apical extremities show a wide range of global 136 137 charges (compare for example T4P and FPE secretins in figure 2), which could be an evolutionary 138 adaptation to their external environment.

139

140 4. Secretin structures reveal discrete common and specific features

141 <u>4.1. The gated pore-forming domain</u>

142 The pore-forming domain of bacterial secretins is highly conserved and form the OM portal through 143 which secreted substrates or translocated appendages transit to the cell surface. Recent cryo-EM data 144 revealed an atypical organization into a giant 56 to 64 stranded β -barrel, where each of the 14-16 145 subunits contributing a 4-stranded beta sheet (Table 1 and Figure 1). The high-resolution structures 146 also revealed an additional inner barrel formed by 4 β -strand per monomers, with the two upper 147 strands extending towards the center of the cavity, perpendicularly to the barrel wall, a structural feature already suspected in many low-resolution structures of secretins [8, 20, 31]. The convergence 148 149 of their loops in the center of the cavity partially obturates the channel to form an orifice of variable 150 size, depending on the secretin type, but always too narrow to allow the transit of its corresponding 151 molecule. This observation agrees with previous studies of secretin channels pore size and 152 electrophysiology showing a pore with a small opening, akin to regular porins [7, 13, 32, 33]. This 153 structural feature separates the periplasmic chamber from the top cavity and is therefore named 154 central gate (CG) (Figure 1). The gating function of CG is supported by the fact that most of the 155 previously identified mutations in the FPE F1 phage secretin pIV, leading to gain of function growth on maltopentaose, bile salt permeability or antibiotic sensibility [34], mapped to the CG [27]. CGs, most 156 157 often captured under closed conformation in isolated particles, must adopt an alternative open 158 conformation to allow the passage of the different structures secreted or assembled by secretins. Such 159 flexibility is comforted by the observation of a partially open CG and bile salt-sensitive phenotype for the T2SS GspD_{G453A} variant, which harbors a glycine to alanine substitution in the central gate [10]. 160 161 Sequence alignment of secretins show that glycine residues are systematically present at least at one bending point of the CG β -strands. In accord with their high flexibility, these residues would allow the 162 rotation of the CG, which would flip upwards and come close to the β -barrel wall to accommodate the 163 164 secreted or assembled structure. Such opening mechanism has recently been confirmed by the 3D structure of the T3SS secretin InvG in an open conformation with the needle assembled inside (Figure 165 166 1) [22]. Importantly, since CG modification not only results in leaky but also non-functional secretins, 167 passage through the CG is probably not a passive process [34]. Given the structural and functional 168 diversity of the molecules passing through this channel, it is not surprising that this diversity is reflected 169 in the CG primary sequences. It is indeed easy to imagine that to accommodate a static needle (T3SS),

- a folded effector (T2SS), a highly dynamic pilus (T4P) or a bacteriophage (FPE), bacterial secretins must
- display different CGs composition and dynamics. Interestingly, even among T2SS secretins, significant
- sequence divergence exists in CGs that may reflect the exquisite substrate specificity existing between
- different T2SS [10]. However, we still lack biochemical data on the interaction interfaces between CGs
- and substrates to understand mechanistically how CGs function in different types of secretins.
- 175

176 <u>4.2. The N3 RBM domain and its involvement in pore formation</u>

177 Global observation of secretin 3D structures (Figure 1) indicates that the N3 domain, previously 178 associated by sequence homology to the periplasmic N-domains, constitutes the building blocks of the 179 C-module with the pore-forming domain. This structural reallocation of the N3 domain to the C-module 180 confirms previous functional data showing its requirement, in addition to the pore-forming domain, 181 for the assembly of a minimal secretin capable of forming a pore in the OM [13, 35]. The N3 RBM 182 domain has the propensity to form ring-like structures [36]. RBMs are also found outside of bacterial 183 secretins where they also play a central ring-building function, e.g., in the IM ring of the T3SS [37] or 184 the sporulation channel component SpoIIIAG [38]. Since T2SS secretins are independent of the β-barrel 185 assembly machinery (BAM), pre-folded in the periplasm and able to fold spontaneously in vitro [39], it 186 is tempting to speculate that the circular oligomerization of secretin C-module could be driven by the 187 ring-like self-assembly property of their RBM domain [37]. This notion is however challenged by a 188 recent structural study indicating that RBM most likely stabilise the oligomer rather than initiate its 189 oligomerization [40]. Nonetheless, RBM conservation in all secretins, as well as its essentiality for 190 oligomerization, support its reallocation as part of the upper C-module.

191

192 <u>4.3. Secretin biogenesis and its scaffolding S domain</u>

193 The different steps driving a protein initially synthesized in the cytoplasm to its final conformation and 194 location consist in overcoming multiple constraints. The secretins must not only travel to the OM but 195 also adopt a complex channel conformation, allowing the passage of large molecules without 196 perturbing bacterial envelope integrity. Their final destination being beyond the IM, the newly 197 synthetized pro-secretin monomer is taken over in the cytoplasm by the SecB chaperone to be 198 exported in the periplasm through the Sec translocon. Once exported, and after cleavage of the signal 199 sequence, secretin monomers are, most often, taken over by an escort protein called pilotin, which 200 will assist in their delivery and proper insertion into the OM [41]. Pilotin maintains the secretin in an 201 intermediate conformation compatible with its transport to the OM, preventing mis-localization 202 and/or premature oligomerization in the IM, which would be deleterious to cell integrity. Such 203 phenotypes have indeed been observed for T2SS and T3SS secretins in absence of their pilotins [42, 204 43]. Several types of mechanisms have been described for the transport of secretins from the 205 periplasm to the outer membrane [44]. The most common, used by most T2SS and T3SS secretins, 206 involves a lipidated pilotin, itself transported to the OM via the Lol pathway [45]. In this case, secretin 207 transport to the OM is independent of the BAM pathway [46] in agreement with its non-β-barreled 208 OM inserted domain [28] and its capacity to spontaneously insert in artificial vesicles [35]. Alternative 209 OM targeting for secretins have been described including Bam-dependent transport [47], involvement 210 of IM accessory proteins for T4P and T2SS secretins [41, 48], or no external assistance like for the self-211 piloted T2SS HxcQ lipo-secretin [49]. Regarding secretin assembly, the existence of folded periplasmic 212 intermediates adopting different conformations prior to the OM insertion has been proposed [39]. 213 Such process has been observed in many pore-forming proteins [50-52] and also supported by the

214 capacity of secretins to oligomerize outside the OM.

215 T2SS and T3SS secretins localization, assembly and OM insertion are mostly governed by their extreme 216 C-terminal S-domains reviewed by [53]. In the high-resolution structures, these S-domains form a helix-217 turn-helix motif that extends laterally on the exterior of the β-barrel (Figure 1). This supports the notion 218 that S-domains have a key role in efficient secretin assembly and stability in addition to their pilotin-219 binding function. S-domains are necessary and sufficient to ensure targeting and stabilization since the 220 graft of the PuID or InvG S-domains to the S-domain deficient FPE pIV secretin rendered the chimeras 221 functional, dependent on the PulS and InvH pilotins, respectively [54, 55]. S-domains of T2SS pilotin-222 dependent secretins are formed by 2 α -helices, respectively involved in stabilization and pilotin 223 recognition. This structure-function relationship is confirmed by the absence of the second helix in 224 secretins targeting to the OM by an alternative pilotin-independent process [48], or for which no pilotin 225 has yet been identified. This is the case for XcpD of P. aeruginosa [26] for which, another mode of OM

targeting could be considered.

227

228 <u>4.4. The periplasmic N-module composed of several successive N-domains</u>

229 With the N3 RBM domain now considered part of the C-module, the secretin N-module is therefore 230 defined as the portion comprising all the domains N-terminal to the N3 RBM domain (Figure 1). Unlike 231 the C-module whose high-resolution structure allowed the precise positioning of each monomer up to 232 amino acids side chains, the lower resolution of N-modules does not allow a clear identification of the 233 monomers. In the majority of the available structures, the N-domains were only positioned by 234 homology-based building using monomeric crystal structures, thus often leading to steric clashes and 235 therefore questioning the accuracy of the models [9, 10, 26, 27, 30]. Such low resolution of N-modules 236 was systematically seen with the first isolated secretin structures, suggesting the requirement of 237 stabilizing partners, which was confirmed subsequently for complexes between secretins and their IM 238 partners [19, 56]. These structures indicate that the N-module is clearly dissociated from the C-module 239 through an unstructured density (Figure 1). This poorly resolved flexible zone may correspond to the 240 inter-domain region separating the OM-associated C-module and the periplasmic N-module. The 241 flexibility in the inter-domain region could allow for important structural rearrangements, essential for 242 the coexistence of the previously discussed different structural organization and stoichiometry 243 between the C- and N-modules.

244 All bacterial secretins harbour a NO domain at their extreme N-terminus, which is involved in IMcoupling. While NO structure was not resolved in secretin oligomers, it has been determined for 245 246 monomeric truncated variants or oligomers in complex with their IM partners [22, 25, 27, 30, 57, 58]. 247 N0 domains of T2SS, T4P, FPE and to some extent T3SS secretins have a similar structural organization, 248 thus suggesting a conserved packing arrangement among secretins. At the quaternary level, structures 249 obtained in combination with IM partners show that the N0 is oriented perpendicularly to the N-250 domain above it (N1 for T2SS/T3SS, and N3 for T4P) [19, 56], while it is positioned in a parallel fashion 251 in absence of IM partners (Figure 1) [25, 58, 59]. NO domains organization within secretin channels 252 appears to be highly variable even in term of stoichiometry. Inside a specific secretin family such as 253 T3SS, pentadecameric and hexadecameric organization have been proposed [22, 56]. For T2SS, 254 pentadecamers and dodecamers (hexamers of dimers), both physiologically relevant, were reported 255 [19, 25].

In addition to the conserved N0 domain, additional RBM domains are sometimes found between
 N3 and N0. This is the case for T2SS and T3SS secretins N-modules, which display two RBMs (N1 and

N2) and one RBM (N1), respectively. These domains could define the size of the periplasmic chamber
therefore reflecting the nature of the envelope in which they are inserted. For example, in the
thermophilic bacterium *Thermus thermophilus*, the T4P secretin PilQ has an N-module composed of
five stacked rings, including four RBMs all dispensable for complex assembly but essential for function
[60]. This larger N-module could represent an adaptation to the thicker cell envelope of this bacterium.

263

264 <u>4.5. Discovery of an optional top-gate (TG) in a sub-class of secretins</u>

265 As new secretin structures were released (Table 1), common and specific determinants of the different family members have emerged. This was the case for T2SS secretins, for which several structures 266 267 revealed the existence in the Vibrio-type subclass [61] of an additional apical domain called top-gate 268 (TG) or cap-gate (Figure 1) [10]. The TG has a conical structure formed by the convergence of 15 pairs 269 of antiparallel β -strands whose connecting loops are relatively loose, suggesting that the hairpin 270 formed by the TG is a dynamic structure alike the CG. The inner diameter of the TG defines a much 271 narrower opening compared to the large open-edged β -barrels formed by the other subclass lacking 272 TG and called *Klebsiella*-type (Figure 1). Given the directionality of effector transport in T2SS, the TG 273 should rotate outward to open and release the substrate in the extracellular medium. After substrate 274 release, the TG would return to its closed conformation to eventually start a new cycle. The presence 275 of such a structure at the top of some T2SS secretins could represent an additional gasket preventing 276 the entry of toxic molecules. Until now, no other type of secretins displayed obvious TG at their summit 277 (Figure 1 and Table 1), an observation consistent with the absence of a corresponding region in their 278 primary sequences [9].

279

280 **5. Concluding remarks**

281 Structural and functional study of bacterial secretins, which remained a mystery for more than 30 years, has largely benefited from the spectacular advances in structural biology and notably from the 282 283 breakthrough of cryo-EM. This revealed intriguing and sometimes unexpected quaternary features of 284 these giant gated double-barrelled channels – such as the interlacement and intertwining of unrelated 285 secondary and ternary structures of each monomer in gates, barrels or cages - providing a coherent 286 atomic quaternary picture. As we move towards a better mechanistic understanding of bacterial 287 secretins, several important challenges remain, including how secretins are transported, assembled 288 and inserted in the OM, how they open and close without compromising their remarkable stability. 289 We need to understand how these giant pores are motioned in the global secretion/assembly context 290 of their corresponding nanomachines, particularly for their poorly resolved N-terminal regions. The 291 ultimate goal - visualizing, at atomic scale, the secretins in their natural environment the bacterial 292 envelope – awaits a revolution in cryo-electron tomography allowing atomic resolution to be reached.

293

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302

303 **Conflict of interest.**

304 None

305

306 Contributions

B. Barbat, B. Douzi and R. Voulhoux contributed to the design and the writing of the manuscript, B.Barbat designed the figures and R. Voulhoux supervised the work.

309 Figures & Table legends

310 Figure 1. Representative cryo-EM structures for each secretin type. The PDB structures have been 311 scaled, aligned with the outer membrane (shaded orange rectangle), and colored according to the 312 following code. Red, monomer; gray, C-module (C+N3 domains); blue, N-module (N0±N1±N2 313 domains); green, S-domain; pink, PulS pilotin; orange, PulC IM-partner. (Top) Side view. The missing 314 NO domain is represented by a shaded blue cylinder. The two different Vibrio (V) and Klebsiella (K) types of T2SS secretins [61] are presented, as well as the "closed" (C) and "open" (O) states of the InvG 315 T3SS secretin. (Bottom) Cross-section view highlighting top-gate (TG) and central-gate (CG) 316 317 constrictions, as well as internal diameters. The disordered loops in N3 domains are indicated by *. 318 Dimensions (in Å) are from measurements on the PDB entries.

Figure 2. Charge distribution of internal cavities in different secret in types. The vertical cross-section
 of the secret ins presented in Figure 1, colored according to their inner surface charge using the APBS
 Electrostatics Plug-in available in PyMol [29]. The color r scale is shown on the left. The position of N3
 domains is indicated by a grey bar.

Table 1. List of cryo-EM 3D structures of bacterial secretins at near-atomic resolution. Secretins for which a near-atomic resolution structure (<7Å) has been published is listed. For each structure, the name of the secretin and its organism of origin, its PDB entry, the stoichiometry, domains that are resolved and the overall resolution are indicated. When useful, additional information on specific features of the structure is provided.

328

329 References

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System	PDB entry	Secretin name	Organism	Domains	Resolution	Secretin's Stoichiometry	Remarks (in complex with)	Reference
T2SS	5WLN	ХсрD	P. aeruginosa	S/C/N3/N2	3.57 Å	15		[26]
	6I1X	ExeD	A. hydrophila	S/C/N3/N2/N1	<i>3.7</i> Å	15		[48]
	5W68	GspD	E. coli EPEC	S/C/N3	<i>3.3</i> Å	15	Top Gate	[28]
	5ZDH	GspD	E. coli ETEC	S/C/N3/N2/N1	<i>3.2</i> Å	15	Top Gate (AspS)	[62]
	5WQ7	GspD	E. coli K12	S/C/N3/N2/N1	<i>3.04</i> Å	15 & 16		[10]
	5WQ8	GspD	V. cholerae	S/C/N3/N2/N1	<i>3.26</i> Å	15	Top Gate	[10]
	5WQ9	GspD _{G453A}	V. cholerae	S/C/N3/N2/N1	<i>4.22</i> Å	15	Partially open state	[10]
	6I1Y	EpsD	V. vulnificus	S/C/N3/N2/N1	<i>3.4</i> Å	15	Top gate	[48]
	6HCG	PulD	K. pneumoniae	S/C/N3/N2/N1/N0	<i>4.3</i> Å	15	(PulCS)	[19]
T3SS	6DV3	InvG	S. enterica	S/C/N3/N1/N0	<i>4.1</i> Å	15	Open state	[22]
	6DV6	InvG	S. enterica	S/C/N3	<i>3.9</i> Å	15	Open state	[22]
	6PEE	InvG	S. enterica	S/C/N3	<i>3.42</i> Å	15		[56]
	6PEP	InvG	S. enterica	N1/N0	<i>3.8</i> Å	16	(SpaPQR:PrgHIJ)	[56]
	6PEM	InvG	S. enterica	N1/N0	<i>3.5</i> Å	16	(SpaPQR:PrgHK)	[56]
	6Q14	InvG	S. enterica	S/C/N3/N1/N0	<i>3.8</i> Å	15 (S/C/N3) 16 (N1/N0)	(SpaPQR:PrgHK)	[56]
	6Q15	InvG	S. enterica	S/C/N3/N1/N0	<i>5.15</i> Å	15 (S/C/N3) 16 (N1/N0)	Open state (SpaPQR:PrgHIJK)	[56]
	6Q16	InvG	S. enterica	N1/N0	<i>4.1</i> Å	16	(SpaPQR:PrgHIJK)	[56]
	5TCQ	InvG	S. enterica	S/C/N3	<i>3.6</i> Å	15		[9]
	5TCR	InvG	S. enterica	S/C/N3/N1/N0	<i>6.3</i> Å	15	(PrgHK)	[9]
	7AHI	InvG	S. enterica	S/C/N3/N1/N0	<i>3.3</i> Å	15 (S/C/N3) 16 (N1/N0)	(SpaPQR:PrgHIJK: SptP)	[24]
	7AH9	InvG	S. enterica	S/C/N3/N1/N0	<i>3.3</i> Å	15 (S/C/N3) 16 (N1/N0)	(SpaPQR:PrgHIJK: SptP)	[24]
	6RWK	MxiD	S. flexneri	N1/N0	<i>3.86</i> Å	16	(MxiG)	[23]
T4P	6VE2	PilQ	P. aeruginosa	C/N3	<i>4.3</i> Å	14	(TsaP)	[21]
	6VE3	PilQ	P. aeruginosa	C/N3	<i>4.3</i> Å	14		[21]
	6VE4	PilQ	P. aeruginosa	C/N3	<i>6.9</i> Å	15		[21]
	6W6M	PilQ	V. cholerae	C/N3/N0	2.7 Å	14		[30]
FPE	70FH	pIV	F1 filamentous bacteriophage	C/N3	2.7 Å	15		[27]

Table1

Secretins for which a near-atomic resolution structure (<7Å) has been published is listed. For each structure, the name of the secretin and its organism of origin, its PDB entry, the stoichiometry, domains that are resolved and the overall resolution are indicated. When useful, additional information on specific features of the structure is provided.

Figure 1



Figure 2

