Identification and structure of an extracellular contractile injection system from the marine bacterium *Algoriphagus machiponganensis*

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Contractile injection systems (CISs) are phage tail-like nanomachines, mediating bacterial cell–cell interactions as either type VI secretion systems (T6SSs) or extracellular CISs (eCISs). Bioinformatic studies uncovered a phylogenetic group of hundreds of putative CIS gene clusters that are highly diverse and widespread; however, only four systems have been characterized. Here we studied a putative CIS gene cluster in the marine bacterium *Algoriphagus machiponganensis*. Using an integrative approach, we show that the system is compatible with an eCIS mode of action. Our cryo-electron microscopy structure revealed several features that differ from those seen in other CISs: a ‘cap adaptor’ located at the distal end, a ‘plug’ exposed to the tube lumen, and a ‘cage’ formed by massive extensions of the baseplate. These elements are conserved in other CISs, and our genetic tools identified that they are required for assembly, cargo loading and function. Furthermore, our atomic model highlights specific evolutionary hotspots and will serve as a framework for understanding and re-engineering CISs.

In most ecological settings, bacteria do not exist as isolated cells, but interact with other organisms. These cell–cell interactions are often mediated by macromolecular machines that translocate effector proteins into the medium or directly into a target cell. Bacterial contractile injection systems (CISs) mediate cell–cell interactions between bacterial and eukaryotic cells and often confer competitive advantage in different environmental niches. CISs are macromolecular injection devices with an overall structure that is homologous to the contractile tails of bacteriophages. Their conserved modules include an inner tube, a contractile sheath and a baseplate complex. For firing of the extended apparatus, the baseplate undergoes a conformational change and triggers sheath contraction, which in turn causes the inner tube to be expelled and injected into a target.

On the basis of distinct modes of action, bacterial CISs are classified into extracellular CISs (eCISs) and type VI secretion systems (T6SSs). eCISs resemble headless phage particles that are assembled in the bacterial cytoplasm and then released into the medium upon cell lysis. Upon binding to a target cell via tail fibres, eCISs contract and puncture the target cell’s envelope. By contrast, T6SSs remain intracellular and are anchored to the inner membrane, injecting effectors by a cell–cell contact-dependent mechanism.

Classical T6SSs (subtypes i–iii) and R-type pyocins (eCISs) form relatively homogeneous groups of CISs. Recent bioinformatic analyses revealed an additional phylogenetic group of CISs with high abundance and diversity. This group comprises hundreds of putative CIS gene clusters, with only a few of them being studied so far. A comprehensive bioinformatic study suggested that these CISs cluster into six distinct phylogenetic clades (Ia, Ib, and IIa–IId). Characterized representatives are often found in clades Ia and Ib and include *Photobacterium virulence cassettes* (PVCs), ‘antifeeding prophages’ (AFPs) from *Serratia*, ‘metamorphosis-associated contractile structures’ (MACs) from *Pseudoalteromonas luteoviolacea*, and the ‘T6SS subtype iv’ (T6SS iv) in *Candidatus Amoebophilus asiaticus*. While PVCs and AFPs in clade Ia act as individual, pyocin-like eCISs with insecticidal functions, MACs and T6SS iv in clade Ib were shown to have diverse functions. MACs form sea-lion arrays of ~100 tethered eCISs that induce metamorphosis of larvae of the marine tubeworm *Hydroides elegans*, and additionally kill insect cells as well as marine macrophages in vitro. The T6SS iv probably mediates the escape of symbiotic *Amoebophilus* bacteria from the phagosomes of its amoeba host.

Given the co-existence of systems with different modes of action and diverse targets in clade Ib, an atomic model of such a CIS assembly would be particularly insightful. The complicated superstructures in MACs and T6SS iv, however, impede their structural characterization. Current high-resolution structural information is limited to clade Ia, that is, PVC and AFP.

Here we performed structural and mechanistic studies on a clade Ib CIS gene cluster in the marine bacterium *Algoriphagus machiponganensis* PR1. This strain was previously co-isolated with the choanoflagellate *Salpingoeca rosetta* and was shown to induce the formation of multicellular colonies (rosettes) of *S. rosetta*.

Bioinformatic analyses showed that ~69% of bacteria in the *Algoriphagus* genus harbour a putative CIS gene cluster.

**Results and Discussion**

A gene cluster in *A. machiponganensis* encodes an eCIS. We set out to characterize a putative CIS gene cluster in *A. machiponganensis* (hereafter referred to as AlgoCIS) that comprised 18 predicted open reading frames (accessions ALPR1_12680-12765; A3HTA7-A3HTC4). Within these open reading frames, we found sequence
similarities with potential CIS structural components, besides additional proteins with unknown functions. Guided by sequence similarities to other CISs, we re-labelled the genes as alg1-18 according to the homologues in Afp (Fig. 1a and Supplementary Table 1). Since there is no homologous protein of ALPR1_12705, a putative tail fibre with Ig-like folds, we labelled it alg19. Previous genome analyses classified CISs into six subtypes, with AlgoCIS being classified as Ib along with MACs and T6SS (ref. 18). This was further supported by our phylogenetic analyses (Fig. 1b).

To explore whether A. machipongonensis expresses any AlgoCIS particles, crude sheath purifications were performed and imaged by negative-stain transmission electron microscopy (EM). Typical CIS-like particles were found in both extended and contracted states (Extended Data Fig. 1a). Subsequent mass spectrometry (MS) analyses of the same sample detected 14 proteins encoded in the AlgoCIS gene cluster (asterisks in Fig. 1a and Supplementary Table 2). To further confirm these results, we disrupted the AlgoCIS gene cluster by inserting a plasmid into the AlgoCIS operon (AlgoCIS-). Since no CIS-like particles were detected in AlgoCIS- mutant (Extended Data Fig. 1b and Supplementary Table 2), we conclude that the AlgoCIS gene cluster encodes CIS-like complexes.

To observe AlgoCIS in a cellular context, we imaged A. machipongonensis cells by cryo-electron tomography (cryoET). We frequently observed AlgoCIS particles in the bacterial cytoplasm but never anchored to the membrane (Fig. 1c), which is incompatible with T6SS mode of action. CryoET analyses and western blots of bacteria at different optical densities (OD600) indicated that the average expression level was highest (~3.8 AlgoCISs per cell) at high OD600 growth phases (Extended Data Fig. 1c,d).

We further analysed the supernatants from wild-type and AlgoCIS− cultures by western blot, negative-stain EM and MS. Using antibodies against Alg1 (inner tube) and Alg2 (sheath), we detected AlgoCIS in the supernatant of a wild-type culture (Extended Data Fig. 1e). We could also observe AlgoCIS particles by negative-stain EM (Extended Data Fig. 1f) and detected most putative structural components in the supernatant via MS (Supplementary Table 2). Taken together, these results indicate that AlgoCIS is consistent with an CIS mode of action.

Overall structure and unique features of the AlgoCIS particle. To gain insights into the general structure of AlgoCIS, purified particles were imaged by cryoET. Sub-tomogram averaging showed the AlgoCIS particle is ~130 nm long and ~30 nm wide, with the tube lumen filled with densities (Extended Data Fig. 1g,h). The structure can be divided into three modules: cap, sheath tube and baseplate. All three modules have 6-fold symmetric features (Extended Data Fig. 1i).

To reveal molecular high-resolution details, we imaged purified AlgoCISs for cryoEM single particle analysis and processed them as was previously shown for other CISs (Extended Data Fig. 2a). The quality of the final maps from the three modules allowed for de novo structural modelling (Extended Data Fig. 2b,c). The sheath subunits are interwoven with densities in the entire sheath layer (Alg2-L1–23) (Extended Data Fig. 2c). The different sheath layers, Alg2 exhibits pronounced structural variations. Both the N and C termini of Alg2 have different conformations in the proximal (Alg2-L1–3) and central sheath layers (Alg2-L1–22) (Extended Data Fig. 2d). The hinge angles of domain IV were also observed to be different when comparing the different Alg2 layers. The domain IV in the distal Alg2-L1–3 layer has the smallest hinge angle, with its tip close to domain II from the neighbouring subunit in the same sheath layer (Extended Data Fig. 2f).

Cap and cap adaptor proteins terminate AlgoCIS. The distal AlgoCIS end is terminated by a hexameric cap complex of Alg16A, and – in contrast to other known eCISs – a hexameric complex of Alg16B, hereafter referred to as the ‘cap adaptor’ (Fig. 2a). The dome-shaped Alg16A cap complex covers the inner tube, and it exhibits an ~11-Å-wide central channel (Fig. 2a and Extended Data Fig. 2a). Each Alg16A subunit interacts with three Alg1 proteins from the distal inner tube layer (Alg1-L) and terminates the tube (Extended Data Fig. 2b). Compared with homologues in PVC/AFP, Alg16A is much shorter (197 amino acids in Alg16A, 293 in Pvc16, 295 in Afp16) and folds into a single domain with a C-terminal extension. Structural superposition showed that the Alg16A structure is similar to the N-terminal domains (NTDs) of Pvc16 and Afp16 (Extended Data Fig. 2c).

Interestingly, the cap adaptor Alg16B mediates the interactions between the cap complex Alg16A and the most distal sheath layer (Alg2-L3). The cap adaptor expands the outer diameter of the cap to ~164 Å, with an enlarged inner diameter of ~115 Å (Fig. 2a). Alg16B NTD (residues 1–213) is an immunoglobulin-like domain flanked by three additional α-helices (α1–3), whereas one single β-barrel constitutes a C-terminal domain (CTD) (residues 214–284) (Fig. 2b). Alg16B adopts a parallel arrangement with Alg16A (Extended Data Fig. 2d). The N terminus of Alg16B interacts with the C terminus of Alg16A in a mimicked ‘handshake’ manner, terminating the sheath assembly (Fig. 2c).

Next we set out to explore the role of the cap adaptor. Due to the lack of available precise genetic tools in A. machipongonensis, we developed a genetic toolset (see Methods and Extended Data Fig. 4e,f) to generate clean in-frame deletions (see Methods and Extended Data Fig. 4e,f). We thus created an AlgoCIS ΔAlg16B deletion and showed by cryoET imaging that it assembled different classes of aberrant AlgoCIS particles (Fig. 2d). Of the particles, ~49.8% (% of ~223) had an overall similar shape compared with the wild type; however, sub-tomogram averaging showed that the cap, cap adaptor and the distal sheath layer (Alg2-L3) were all absent (Fig. 2e). Another ~36.8% of particles contained only the inner tube and the baseplate, which is similar to the previously reported tube-baseplate complexes (TBC). Additionally, ~13.4% were TBC complexes with contracted sheath, similar to the ‘contracted with jammed tube’ particles seen in MACs3. Moreover, the tube lumen was empty for all observed particles in AlgoCIS ΔAlg16B (Fig. 2d and Supplementary Table 2). Together, these results showed that the cap adaptor plays an important role in stabilizing the cap module and the most distal sheath layer, and its absence results in mis-assembled particles.

The sheath-tube module reveals diverse structural conformations across sheath layers. Six protofilaments of sheath proteins adopt a right-handed helical array with a length of 92.4 nm and an outer diameter of 24.6 nm (Fig. 1d). Unlike the multiple sheath proteins present in PVC/AFP, the AlgoCIS sheath is composed of only one protein, Alg2, which folds into four domains (Extended Data Fig. 5a,b). The conserved domains I and II contribute to the sheath wall, with additional domains III and IV extending outwards (Extended Data Fig. 5c). The sheath subunits are interwoven with each other via a conserved ‘handshake’ and iteratively assemble into the full sheath, where the attachment helix in domain I mediates the interactions with the inner tube (Extended Data Fig. 5d,e).

Interestingly, depending on its location in the different sheath layers, Alg2 exhibits pronounced structural variations. Both the N and C termini of Alg2 have different conformations in the proximal (Alg2-L1–3) distal (Alg2-L1–3) and central sheath layers (Alg2-L1–22) (Extended Data Fig. 5f). The hinge angles of domain IV were also observed to be different when comparing the different Alg2 layers. The domain IV in the distal Alg2-L1–3 layer has the smallest hinge angle, with its tip close to domain II from the neighbouring subunit in the same sheath layer (Extended Data Fig. 5f,g). The different
Fig. 1 | Identification and characterization of a contractile injection system in A. machipongonensis. a, Schematic showing the gene cluster of a putative contractile injection system in A. machipongonensis (Alg0CIS). The genes are labelled on the basis of similarities to AFP. Gene products that were detected by MS are marked by asterisks. The gene accession numbers are shown above the corresponding genes. b, Phylogenetic analyses based on putative sheath proteins showing that the closest relatives of Alg0CIS are MACs and T6SSii, which belong to clade Ib CIS. The different representatives are colour-coded on the basis of their modes of action. c, Representative cryoET slice of A. machipongonensis cell (left) and the corresponding model (right), showing cytoplasmic Alg0CISs that are not attached to the inner membrane. CP, bacterial cytoplasm; IM/white, inner membrane; OM/pink, outer membrane; green, Alg0CIS particle. One representative Alg0CIS particle is marked with a white box. Shown is a 10.8 nm thick slice. Scale bars, 50 nm. In total, 38 tomograms were acquired. d, Shadowed surface (left) and ribbon (right) diagrams showing the overall cryoEM structure of Alg0CIS in the extended state (sliced views in the centre). Structural subunits are colour-coded according to the gene cluster in a. e, Perpendicular views of shadowed surface and ribbon diagrams showing the Alg0CIS model corresponding to the sections in d.
conformations of N and C termini in the most proximal (Alg2-L1) and most distal sheath layers (Alg2-L23) might mediate the assembly of the sheath-tube module. A lowpass-filtered map indicates that there are two potential conformations of domain IV in the central sheath layers (Extended Data Fig. 5h).

Regarding the inner tube, the 22 hexameric layers (Alg1-L1–22) arrange in the same helical parameters as the extended sheath, forming a 90.2-nm-long conduit with a 3.6-nm-wide tube lumen (Fig. 1d). The inner tube subunits possess the conserved β-barrel structures and the N terminus of Alg1 is shorter than that of Pvc1 and Afp1 (Extended Data Fig. 5i). The inner surface of the tube is negatively charged as in other CISs24,25 (Extended Data Fig. 5j). The structures of the tube initiator and central spike reveal unique domain organization. The inner tube is attached to the central spike by the tube initiator complex (Alg5/Alg7), which is further docked onto the spike (Alg6/Alg8/Alg10) (Fig. 3a). All components of the tube initiator complex possess the conserved β-barrel folds seen in Alg1 (Extended Data Fig. 6a), whereas Alg7 has one additional C-terminal LysM domain extending out and interacting with the peripheral wedges (Fig. 3a and Extended Data Fig. 6a).

Surprisingly, the linker between the β-barrel and LysM domain in Alg7 was found to be cleaved (Extended Data Fig. 6b).

The spike is located below the tube initiator complex and comprises three intertwined copies of Alg8 (Fig. 3a). The N-terminal part of Alg8 forms a gp27-like domain and functions as a 3-to-6-fold symmetry adaptor35. The gp27-like domain is followed by the mid β-barrel domain and C-terminal gp5-C-like domain, which are homologous to T4 gp5 (Fig. 3b). Intriguingly, the gp5-C-like and mid β-barrel domains in Alg8 twist an additional 120° around the central axis and are much longer when superposing the gp27-like domains against homologues in PVC/AFP (Fig. 3c). The tip of the central spike binds to a single copy of Alg10, which is a proline–alanine–alanine–arginine (PAAR)-like protein (Fig. 3a).

The Alg6 plug protein is crucial for CIS assembly and function. Interestingly, our symmetry-relaxed map showed an additional prominent density plugged into the tube-exposed cavity of the spike. We identified this density by structural modelling unambiguously as a trimer of the uncharacterized protein Alg6 (Fig. 3d and Extended Data Fig. 6c), hereafter referred to as the ‘plug’. Alg6 is an orthologue of Afp6, which was previously not resolved25. Alg6 includes an
N-terminal single strand and a C-terminal α-helix, which are linked by a flexible loop (Extended Data Fig. 6c). The Alg6 N terminus extends along the inner surface of the Alg8 gp27-like domain, then folds back and forms the C-terminal α-helix (Fig. 3d). The hydrophobic core of Alg6 contributes to the trimeric assembly, directing the hydrophilic surface to interact with other baseplate components (Fig. 3d).
There are extensive contacts between Alg6, Alg8 and Alg7 (Extended Data Fig. 6d,e), suggesting that Alg6 is essential and functions as a nucleus for particle assembly. To test this hypothesis, we generated and analysed a ΔAlg6 mutant. Remarkably, no assembled Alg6-CIS particles were found in a sheath preparation of the knockout (Extended Data Fig. 6f). We also detected putative ‘plug’ homologues in the closely related MACs, as well as in the T6SSα (accessions JF50-12690/Aasi_1078) (Extended Data Fig. 6g). We deleted the plug homologue in MACs in P. luteoviolacea assembly and it interacts extensively with other baseplate components (Extended Data Fig. 7e).

Conserved baseplate components with downward extending tail fibres. The central spike is surrounded by a hexagonal iris-like ring of Alg11-Alg12 heterodimers, forming the baseplate ‘wedges’ with the conserved core bundle and trifacturation units (Fig. 4a). Six copies of the gp25-like protein (Alg9) attach above Alg11-Alg12, where the C termini from two neighbouring protomers were observed to be slightly different (Extended Data Fig. 7a). Like gp25 in the T4 phage23, Alg9 mimics the ‘handshake’ to initiate sheath assembly and it interacts extensively with other baseplate components, implying a role in the initiation of contraction (Fig. 4a and Extended Data Fig. 7b).

Similar to Pvc12 and Afp12, Alg12 contains gp6-like and gp7-like parts (Extended Data Fig. 7c). Domains I–III constitute the gp6-like part and participate in the assembly of the wedges, leaving the gp7-like part (domains IV–VII) exposed on the lateral surface of the baseplate (Fig. 4a). Three-dimensional-focused classification revealed diverse conformations for the gp7-like part of Alg12 (Extended Data Fig. 7d). It is responsible for interactions with tail fibres. In contrast to AFP/PVC12, the Alg6-CIS tail fibres do not fold back to contact the sheath, but instead attach on the lateral surface of the wedges and extend downwards (Fig. 1d). CryoEM 2D projection images showed that tail fibres adopt various conformations (Extended Data Fig. 7e).

Alg11 extensions form a unique spike cage. AlgCIS exhibits a remarkable structural feature on the baseplate—the formation of an extensive ‘cage’ around the central spike. Therefore, Alg11 is much longer and folds into six domains compared with the homologues (Fig. 4b). The Alg11 domain I forms the conserved core bundle together with the domains I and IV in Alg12 (Fig. 4a). The Alg11 N terminus extends across the cleft between domain I and VI (Fig. 4b and Extended Data Fig. 7f). Interestingly, there is one additional large domain (IV, residues 278–643) protruding from the distal tip of domain III (Fig. 4b). The domains IV from the six protomers form a hexagonal cage that surrounds the spike (spike cage). The outer surface of the spike cage is negatively charged, with one positively charged residue at the tip (Lys572) (Fig. 4c and Extended Data Fig. 7g). The lumen of the spike cage ranges in diameter from 56 Å to 18 Å and a tip cavity is filled by Alg10 (Fig. 4c).

Conformational changes of the sheath and baseplate cage after contraction. To explore the mechanism of AlgoCIS firing, we determined post-firing structures of both sheath (Extended Data Figs. 2b, 3c,d and Supplementary Fig. 1b) and baseplate (Supplementary Fig. 2). Similar to other CISs24,25,40,41, the displacement of N and C termini of the sheath subunits result in a similar rigid-body rotation of the sheath subunits, leading to sheath contraction (Supplementary Fig. 3).

In the contracted state, the spike cage adopts an open conformation, with the inner tube expelled across its centre (Extended Data Fig. 8a). The conformational change of the spike cage is mainly attributed to the movements of domain IV of Alg11, which tilts an additional ~27.4° outwards (Extended Data Fig. 8b). In addition, the tail fibres show a large outward tilt (~57°) (Extended Data Fig. 8c). Strikingly, the overall structure of the tail fibre in the contracted state has a shape that is similar to the T6SS baseplate component TssK (PDB entry: 5MWN)42 (Extended Data Fig. 8d).

Furthermore, upon contraction, the baseplate protein Alg12 has a higher hinge angle with the plane of the iris-like ring (~66° in contracted, ~48° in extended state) (Extended Data Fig. 8e). Interestingly, the iris-like ring structure in AlgoCIS was found intact and having a slight expansion upon contraction (Extended Data Fig. 8a), which is different from the contracted structures in both pyocins and Afp25,30.

Given the conformational changes that were observed in the baseplate components, we speculate that AlgoCIS might employ a similar signal transmission mechanism as the T4 phage (Extended Data Fig. 8f): the large outward tilting of tail fibres causes the...
rotation and outward motion of baseplate components; the signal is subsequently transferred to the Alg11/12 core bundles and contributes to the tilts of the core bundle and the bound proteins; the conformational changes of Alg9 further trigger sheath contraction.

ALPR1_12695 (Cgo1) and ALPR1_12690 (Cgo2) are cargo proteins located in the tube lumen. In addition to the CIS structural proteins, the AlgoCIS gene cluster encodes two DUF4157 domain-containing proteins with unknown functions (Fig. 1a).
We will hereafter refer to these genes as cgo1 and cgo2, respectively. Cgo1/Cgo2 share a similar predicted disordered NTD and a central DUF4157 domain, while their CTDs are different (Extended Data Fig. 9a). Interestingly, DUF4157 domains were previously identified as characteristic effector domains in eCISs and T6SSes. Due to the closely related MACs having an effector/cargo protein that was previously reported to localize to the tube lumen, we set out to determine whether Cgo1/Cgo2 share this cargo role in AlgoCIS.

To test this hypothesis, we generated mutants by abolishing the expression of Cgo1/Cgo2 individually, or both (Extended Data Fig. 9b). CryoET revealed that the wild-type inner tube was almost always filled (~96%, n_total = 2,620) (Fig. 5a and Extended Data Fig. 1j). In contrast, the inner tube was mostly empty (~93%, n_total = 543) in the Cgo1/Cgo2− mutant and the occupancy was dramatically reduced in the individual Cgo1− and Cgo2− mutants (~38%, n_total = 1,383 and ~37%, n_total = 1,104, respectively) (Fig. 5a). The difference map between sub-tomogram averaging volumes of wild-type and Cgo1/Cgo2− mutant shows additional densities in cyan (corresponding to cargo proteins) and the remaining parts in white. Scale bars, 10 nm.

Fig. 5 | Cgo1 (ALPR1_12695) and Cgo2 (ALPR1_12690) are cargo proteins filling the tube lumen. a, Top: cryoET slices of purified wild-type AlgoCIS and different deficient mutants showing that the fractions of empty AlgoCIS particles are significantly increased in mutants (wild-type, n_total = 2,620; Cgo1−, n_total = 1,383; Cgo2−, n_total = 1,104). Bottom left: representative filled and empty AlgoCIS particles. Bottom right: quantification (blue, filled; red, empty). The individual empty AlgoCIS particles are highlighted by red arrowheads, whereas one filled AlgoCIS particle is highlighted by a blue arrowhead. Tomographic slices are 10.8 nm thick. Scale bars, 50 nm. b, Central volume slices showing the sub-tomogram averages of wild-type AlgoCIS (left) and Cgo1/Cgo2− (middle). The difference map (right) between wild-type and Cgo1/Cgo2− mutant shows additional densities in cyan (corresponding to cargo proteins) and the remaining parts in white. Scale bars, 10 nm.

Due to our observation that Cgo1/Cgo2 are located in the tube lumen, we explored the protein–protein interactions of the two cargo proteins and determined whether they could be co-loaded into the same tube. Our results indicated that the cargo proteins probably do not specifically interact with one another (Extended Data Fig. 9c). Nevertheless, we could not exclude the possibility that two cargo proteins are loaded into the same tube without strong contacts. Together, we conclude that Cgo1/Cgo2 are two cargo proteins that can be loaded independently from each other into the lumen of the inner tube, from which they are released after contraction.

**Heterologous expression of Cgo1 inhibits bacterial growth.** Sequence analyses predicted that the Cgo1 CTD might encode a metalloprotease (Extended Data Fig. 9a). To further explore a possible function of AlgoCIS, the Cgo1/Cgo2 were recombinantly expressed in *Escherichia coli*. The expression of the full-length Cgo1 protein inhibited growth of the bacteria and the effect of Cgo1 was independent of the metalloprotease motifs in DUF4157 and CTD domains. Furthermore, neither the fusion of a periplasmic translocation tag (Tat system), nor the co-expression of Cgo1/Cgo2 mitigated the inhibiting effect of Cgo1 (Extended Data Fig. 9d).

Besides many CISs having an anti-bacterial effect, some eCISs were reported to mediate interactions with eukaryotic cells. We co-incubated a range of potential eukaryotic targets with...
A. machipongonensis bacteria. The comparison of assays performed with wild-type vs AlgoCIS® mutant bacteria showed no significant differences (Extended Data Fig. 10b). The same was true when we tested the effect of purified AlgoCIS with insect cells—a known target of MACs59 (Extended Data Fig. 10c). Since A. machipongonensis was reported as a potential bacterial prey of the choanoflagellate S. rosetta58, we also hypothesized that AlgoCIS might affect the proliferation of the choanoflagellate. However, no significant effect was detected when the intact bacteria or purified AlgoCIS were incubated with the choanoflagellites (Extended Data Fig. 10b,d).

Conclusions

Besides the classical T6SSs (subtypes i–iii) and pyocins (eCISs), recent bioinformatic genome analyses uncovered a significant additional phylogenetic group of CISs16–18. In addition to their abundance across sequenced bacterial genomes, an intriguing feature of this group is the co-existence of eCISs and T6SSs among very close relatives in clade Ib. The high-resolution structures presented here and in an accompanying study49, will serve as a framework to understand the evolution and function of these related systems. In this regard, our discovery of features such as the cap adaptor, plug and spike cage are significant, since we found that they were conserved in many other systems (Fig. 6a).

Another major insight is the identification of evolutionary hotspots. Our data suggest that the gp7-like part of the baseplate wedge-component Alg12 and its homologues mediate the formation of superstructures (Fig. 6b). In T6SSv, up to 34 individual T6SSv structures form ordered arrays, mediated by lateral interactions of the baseplates16. In MACs, ~100 individual eCISs form ordered arrays, mediated by long tail fibres that connect neighbouring eCISs22. The structural comparisons enabled by the AlgoCIS structure presented here suggest that the gp7-like baseplate components are probably involved in mediating these interactions – directly in T6SSv and by binding the long tail fibres in MACs (Fig. 6b, cyan). AlgoCISs, AFPs and PVCs are thought to act as individual eCISs and the gp7-like baseplate component (Fig. 6b, cyan) mediates contacts with different types of tail fibres.

The AlgoCIS tail fibres themselves reveal a surprising degree of compositional and structural heterogeneity among related assemblies. No homologues of Alg19 were found in AFP/PVC or MACs, which feature different types of tail fibres that emanate from the baseplate and are oriented towards the distal end/sheath. Alg19, however, rather adopts an orientation downwards, with the general structure being found to be similar to the corresponding region in the canonical T6SS baseplate component TssK.

A second evolutionary hotspot that is revealed by the comparison of the AlgoCIS structure with sub-tomogram averages of MACs and T6SSv is a binding site at the cage domain of Alg11 homologues (Fig. 6b, orange). This site mediates the attachment of short tail fibres in MACs and the attachment of the entire apparatus to the inner membrane in T6SSv. Eventually, our data may enable structural and functional predictions for uncharacterized CIS gene clusters in the future.

Finally, our genetic tools for A. machipongonensis enable future approaches to explore the molecular details of AlgoCIS assembly, signal transmission and contraction in the natural host organism. The combination of the AlgoCIS structure, together with another concurrently reported system in cyanobacteria22, the sub-tomogram averages of other clade Ib CISs, and the genetic tools will facilitate targeted approaches to re-engineer AlgoCIS for biomedical applications and may even allow for switching its mode of action.

Methods

Sheath preparation of AlgoCIS. The AlgoCIS purification was followed as previously reported with some modifications42. Briefly, a small volume of A. machipongonensis was inoculated into 1 l fresh marine broth (MB) medium (Condalab) and grown at 30 °C and 200 r.p.m. for 2 d (bacterial OD600 = 5.0). The additional antibiotic erythromycin was added to the medium at the final concentration of 50 μg ml⁻¹ for culturing deficient mutant strains. The bacterial pellet was harvested by centrifugation and resuspended with buffer A (20 mM Tris pH 8.0, 150 mM NaCl, 50 mM EDTA). The lysis reagents (1% Triton-X100, 0.5x Celllytic B (Sigma-Aldrich), 200 μg ml⁻¹ lysiszyme, 50 μg ml⁻¹ DNAse I, and protease inhibitor cocktail (Roche)) and 1.5 mM final concentration of MgSO4 were added into the bacterial suspension and incubated at 37 °C for 30 min to lyse the bacteria. The cell debris were removed by centrifugation at 15,000 g and 4 °C for 20 min. The supernatant was subjected to ultra-centrifugation with sucrose cushion (1 ml at bottom) (20 mM Tris pH 8.0, 150 mM NaCl, 50 mM EDTA, 1% Triton-X100, 50% (w/v) sucrose) at 150,000 g and 4 °C for 1 h. The sucrose cushion was taken, together with some remaining overlying liquid (~ 0.5 ml). The residual bacterial contamination in solution was further removed by centrifugation at 15,000 g for 15 min. The sample was subjected to a second
round of ultra-centrifugation without a sucrose cushion. The resulting pellets were washed, soaked with buffer B (20 mM Tris pH 7.5 and 150 mM NaCl) overnight, and then resuspended. The crude samples were subjected to negative-stain EM imaging and mass spectrometry.

For cryoEM analysis, the crude sample was further purified through a 10–50% (w/v) sucrose gradient at 100,000 g for 4 h using the SW 55 Ti rotor. The gradient was divided into 11 fractions and each fraction was checked for AlgCIS with negative staining EM. The fractions containing AlgCIS were diluted with buffer B, passed through a 0.1-μm-pore filter twice, and then concentrated by a third round of ultra-centrifugation. The pellets were resuspended in buffer B and filtered through 0.22 μm centrifugal filtering (Millipore).

The contraction of AlgCIS was performed following a previously reported method. The purified AlgCIS samples were incubated with 2 M guanidine-HCl at room temperature for 0.5 h. The guanidine-HCl was replaced by buffer B using Slide-A-Lyzer MINI dialysis devices (ThermoFisher). The contracted samples were subjected to cryoEM vitrified sample preparation. To separate the released cargo proteins from contracted AlgCIS, the samples were ultra-centrifuged at 150,000 g and 4 °C for 1 h. The pellets were resuspended in buffer B.

AlgCIS purification from bacterial supernatant. The purification of released AlgCIS from the bacterial supernatant was performed using the (NH₄)₂SO₄ precipitation method. Briefly, different A. michiganensis strains (wild type, AlgCIS⁻ and null mutant) were inoculated in 50 ml MB broth and incubated at 30 °C, shaking at 200 c.p.m. for 2 d. The bacterial cells were centrifuged at 7,000 g for 20 min and the supernatant was taken for subsequent purification. The saturated (NH₄)₂SO₄ solution was slowly added into the supernatant to a final concentration of 1.4 M and kept stirred at 4 °C overnight. The precipitated pellets were washed by centrifugation at 12,000 g and 4 °C for 1.5 h. The pellets were resuspended using buffer B and filtered via 0.22 μm centrifugal filtering. The purified samples were analysed by negative staining EM, mass spectrometry and western blotting.

In the western blotting, the rabbit polyclonal antibodies against Alg1 (inner tube protein, generated from GenScript) or Alg2 (sheath protein, generated from GenScript) were respectively diluted to the final concentration of 1 μg ml⁻¹, while the rabbit polyclonal antibody against recA (abcam, ab63797) was regarded as the loading control and was diluted at the ratio 1:2,000.

Mass spectrum analysis. The purified AlgCIS samples were sent in solutions to the Functional Genomics Center Zurich (FGCZ), which performed the mass spectrum and the subsequent data analysis. The samples were first digested by trypsin. These digested samples were dried and dissolved in 20 μl ddH₂O with 0.1% formic acid. The samples were transferred to autosampler vials for liquid chromatography–mass spectrometry analysis (LC–MS/MS). The samples were diluted at the ratio 1:40, with 1 μl of each sample being injected on a nanoAcquity UPLC coupled to a Q-Exactive mass spectrometer (ThermoFisher).

The acquired MS data were converted to a Mascot Generic File format and were processed for identification using the Mascot search engine (Matrixscience). In addition, the acquired MS data were input into PEAKS Studio (Bioinformatic Solutions) and were searched against the Algoriphagus michiganensis database. The results were visualized by Scaffold software.

Vitrified sample preparations. The purified AlgCIS particles were vitrified on 200 mesh Quantifoil Gold grids (R 2/2) using a Vitrobot Mark IV (FEI company), whereas the contracted AlgCIS particles were vitrified on Quantifoil Gold grids (R 2/2) coated with 1 nm thickness of carbon layer. For cryoET, different samples (purified wild-type AlgCIS and related mutants, wild-type bacteria at different OD₅₆₀, solutions were seeded with 10 nm BSA-coated colloidal gold particles at the ratio 1:5 before application to EM grids. The vitrified samples were first checked using a Tecnai F20 microscope (ThermoFisher) operating at 200 kV. The grids with appropriate ice thickness and good particle distribution were used for subsequent data collection.

Samples of MACs and A. asiaticus cells were prepared as previously described. The samples were mixed with Protein A-conjugated 10 nm colloidal gold before plunge freezing using Quantifoil Copper grids (R2/1).

CryoET data collection and tomogram reconstruction. CryoET datasets of wild-type AlgCIS and bacteria at different OD₅₆₀ were collected as movie stacks at a nominal magnification of 33,500 (an effective pixel size of 2.68 Å with a defocus range of −10 to +60 μm) on a Titan TF Krios EM (ThermoFisher) operating at 300 kV and equipped with an energy filter and a K3 Summit detector (Gatan). The tilt series of purified AlgCIS mutants (AlgCIS Cgo1/Cgo2⁻, Cgo1⁻, Cgo2⁻, and ΔAlg16B) were collected with the same biocylindrical scheme at a magnification of 33,500 (an effective pixel size of 2.75 Å) on a Titan Krios EM operating at 300 kV and equipped with an energy filter and a K2 Summit camera (Gatan). All datasets were collected at a defocus value of ~−8 μm. The dose rate of each tilt was −2.1 eÅ⁻² and the total dose was ~130 eÅ⁻². The tomograms were aligned, reconstructed and segmented using the IMOD programme suite. The contrasts of some tomograms were further enhanced using the deconvolution filter ‘om_deconv’.

The structural determination of the homologue PVC model (EMDB entry: 9763), and was used to generate the 'helical reconstruction' mode without imposing helical parameters, where the Contrast transfer function (CTF) estimation was done using Gctf and CTF correction was done by phase flipping in IMOD.

Sub-tomogram averaging of wild-type AlgCIS and mutants. The sub-tomogram averaging of wild-type AlgCIS, Cgo1/Cgo2⁻ and ΔAlg16B was performed with ‘Dynamo’. The individual AlgCIS particles were manually picked using dipole set models from the reconstructed tomograms without CTF correction at a binning factor of 4. The sub-volumes of AlgCIS particles (wild-type: 1,976 from 23 tomograms, Cgo1/Cgo2⁻: 404 from 46 tomograms, ΔAlg16B: 83 from 30 tomograms) were extracted and subsequently averaged. All sub-tomograms were split into half-datasets on the basis of the odd-and-even with ‘dteo’ package in ‘Dynamo’. The half-datasets were individually aligned against the same reference for 2 iterations of coarse alignment and the subsequent 2 iterations of fine alignment, assuming 3-fold symmetry. The averaged sub-volumes from half-datasets were used to estimate the Fourier shell correlation using ‘relic’ postprocess. The final resolutions of averaged sub-volumes of wild-type AlgCIS, Cgo1/Cgo2⁻ and ΔAlg16B were estimated at 44 Å, 30 Å and 33 Å, respectively.

To generate the difference map between the averaged sub-volumes of wild-type and Cgo1/Cgo2⁻ mutant, the two volumes were aligned against each other and lowpass filtered to 45 Å. The difference map was further generated by volume subtraction of the wild-type against the Cgo1/Cgo2⁻ mutant using ‘diffmap’ (https://grigoriefflab.umassmed.edu/diffmap).

Sub-tomogram averaging of the baseplate in MACs and A. asiaticus. Dynamo was used for all sub-tomogram averaging steps. Particles were picked manually (using Pickman in plane orientation and 110 and 66 particles for Macs and A. asiaticus cells, respectively. A total of 816 MACs and 366 A. asiaticus baseplate particles were extracted from tomograms binned by a factor of 4. An initial reference was obtained by averaging all particles using orientations from picking and applying 6-fold symmetry. After initial alignment limited to ±40 °K, the dataset was split into two half-datasets using ‘dteo’ and processed independently. Particles were re-extracted from binned tomograms by a factor of 2 and alignment was further refined. The final resolution of the baseplate in MACs and A. asiaticus was estimated at 11 Å and 29 Å, respectively.

CryoEM single particle data collection and image processing. CryoEM datasets of purified AlgCIS and contracted AlgCIS were collected as movie stacks at a nominal magnification of 81,000 (an effective pixel size of 0.55 Å at super-resolution) using the SerialEM programme on a Titan Krios EM operating at 300 kV and equipped with an energy filter and a K3 Summit camera. The data collection was performed in super-resolution mode and the total exposure time was ~1 s. Each stack contained 50 frames and the accumulated electron dose rate was ~60 eÅ⁻². The movie frames of each collected stack were aligned and summed up into one single micrograph with dose weighting at the binning factor of 2 using MotionCor2 (effective pixel size of 1.10 Å). The CTF parameter of the micrographs were estimated using Gctf. A total of 6,689 micrographs of purified AlgCIS with good defocus value range (~1.8 to ~3.0 μm) and low drift were selected for subsequent image processing, whereas a total of 7,984 micrographs of contracted AlgCIS were used to determine the structure of the baseplate.

The image processing of purified AlgCIS was performed as previously reported. The AlgCIS particles were picked manually using Relion3.0, with the start–end coordinate pairs in arbitrary direction (start point: cap module; end point: baseplate). The particle extraction was performed in ‘Extract helical segments’ mode to extract helical segments of AlgCIS particles on the basis of the arbitrary start–end coordinate pairs. The first segments of AlgCIS particles (128,473 particles), corresponding to the cap, were used for the structural determination of the cap module, whereas the last segments of AlgCIS particles (128,473 particles) were applied to determine the baseplate complex. The middle segments (18,133 particles) were subjected to sub-tomogram analysis. Interested areas of the extended sheath-tube module using helical reconstruction in Relion3.0 (Extended Data Fig. 2a).

For the cap module, the bad particles were first excluded through 2D classification. A total of 65,059 particles were then used for 3D refinement in ‘helical averaging without imposing’ mode with a nominal tilt range, where the prior tilt and psi information of each start–end coordinate pair were applied in the calculation. The initial reference was generated by scaling and lowpass filtering of the homologue PVC model (EMDB entry: 9763), and was used to generate the
The initial helical parameters were deduced from the initial reconstruction. The particles in good classes were first subjected to 3D auto-refinement using images at the binning factor of 1. The particles in the best 3D class (Class VIII) were used for the 3D focused refinement, resulting in a 2.9 Å resolution structure of the baseplate complex (Extended Data Figs. 2a and 3a,b). Briefly, the symmetry equivalent orientations were first generated by adding an additional 60° to \( \text{relAngleRot} \) of individual particles, and combined with the corresponding original orientations. Then, the 3D classification without angular sampling was performed among these combined orientations with a mask around the central spike. The particles with the correct 3-fold orientations were clearly separated in this process and subjected to a local 3D refinement. The final structure of 2.8 Å resolution of the baseplate was reconstructed from 82,696 particles with applied 3-fold symmetry (Extended Data Fig. 3a). For the overall AlgoCIS structure, the centre of the individual baseplate was moved along the z axis (or 3-fold symmetry axis) to the centre of the AlgoCIS particle on the basis of the refined orientation. The AlgoCIS particles were then re-extracted from raw micrographs and the relative orientation of individual baseplates was applied to the corresponding CIS particles. The bad particles were removed through 2D classification and the good particles were applied to a local 3D refinement at the binning factor of 2, assuming 3-fold symmetry. A total of 52,837 particles were used to determine the 4.4 Å structure of the overall AlgoCIS particle (Extended Data Fig. 2a). Although the resolution of the AlgoCIS particle reached the Nyquist frequency at the binning factor of 2, the structure of AlgoCIS was sufficient for the structural assignment of the entire atomic model. In addition, the big box size of particles (1,600 pixels) would occupy a lot of computational resources. Thus, we did not continue the structural refinement against the raw images at the binning factor of 1.

The final 2.5 Å resolution structure of contracted sheath was obtained from 92,922 particles applied 6-fold symmetry (Extended Data Fig. 3b,c,d). For the contracted sheath, the helical particles were first manually picked using star-end pairs in Relion3.0 and we then performed particle extraction in ‘Extract helical segments’ mode. The extracted helical segments were subjected to 2D classification in good classes. Then, the particles were processed as that of the extended sheath-tube module (Extended Data Fig. 2b). The final 2.5 Å resolution structure of contracted sheath was obtained from 92,922 particles applied with 6-fold symmetry and helical parameters (rise = 40.80 Å, twist = 20.54°) (Extended Data Figs. 2a and 3a,b). To determine the baseplate of contracted AlgoCIS, the baseplate particles were manually picked in Relion3.0 (Supplementary Fig. 2a). A total of 21,933 particles were extracted and directly subjected to 3D auto-refinement, assuming 6-fold symmetry with the 60 Å lowpass-filtered baseplate model in contracted AFP (EMDB entry: 4876) at the binning factor of 4. Local 3D classification was applied to the baseplate. The particles from bad 3D classes (class I–III) were subjected to one additional 2D classification without angular sampling and the good particles were combined with the particles in 3D classes IV and V. Then the combined particles were subjected to 3D auto-refinement at the binning factor of 2. The density around the baseplate was further refined by three rounds of focused 3D classification. The particles in three rounds of good classes were combined and the duplicates were removed. A total of 3,793 particles were used to determine the 4.8 Å resolution structure of the baseplate, which was primarily attributed to the map quality of the sheath-tube module. The resolution of the baseplate was estimated via a local mask, which was resolved to 8.9 Å resolution (Supplementary Fig. 2b).

The structural modelling results are listed in Supplementary Table 4. The in-frame deletions of the AlgoCIS genes were used to design the new AlgoCIS mutants. The molecular graphs were made using Chimera and ChimeraX.

### Generation of AlgoCIS mutants

The M. achipongonensis AlgoCIS deficient mutants generated in this paper are all site-directed insertion mutants with pYT313[^1], a bacteriodes suicide plasmid containing an erythromycin-resistant cassette, inserted into the genes of interest. A 2,300 bp long sequence containing the sequence of the gene of interest was cloned into pYT313. The constructed plasmid was then transformed into M. achipongonensis with a modified protocol[^2]. Briefly, a 5 ml overnight bacterial culture was spun down and washed with sucrose buffer (272 mM sucrose, 1 mM MgCl2, 7 mM K2HPO4, pH 7.5) three times and then sucrose buffer containing 15% (v/v) glycerol three times at 37 °C. The cells were then extracted and the sheath-tube module (1 nm-wide) was picked up at a 1-mm-wide Gene pulser electroporation cuvette (Biorad). The electroporation was performed using Gene Pulser Xcell Electroporation System (Biorad) with 1.5 kV, 200 μs capacitance. Fresh MB medium was added and the cells were recovered at 30 °C for 4 h. The recovered cells were plated on marine (MB) agar plates (Condala) containing 50 μg/mL erythromycin to select mutants with pYT313 inserted. All bacterial mutants were further confirmed by colony PCR. For AlgoCIS, 2,300 bp of the nucleotide sequence containing the N-terminal coding region of Alg8 was cloned and inserted into pYT313. For the null mutant, 2,300 bp of a pseudogene (ALPR1_RS19250) was cloned into pYT313 and the insertion of the constructed plasmid into the pseudogene did not disrupt any functional gene in the bacterial genome. For AlgoCIS Cgo1/Cgo2 and individual mutants (Cgo1− and Cgo2−), the 2,300 bp upstream of the related gene, all the downstream genes in the AlgoCIS gene cluster and the erythromycin-resistant cassette, including the corresponding promoter, were synthesized and further constructed into pUC57 vector (produced from Genscript) (Extended Data Fig. 4b). The primer used for AlgoCIS null mutants are listed in Supplementary Table 4.

The in-frame deletions of the AlgoCIS genes were used to design the new AlgoCIS mutants. The assembly plasmid was then electroporated into SM10 E. coli, with the sequence of the assembled product being confirmed via DNA sequencing before being conjugated with M. achipongonensis on MB agar plates and incubated at 30°C overnight. The following day, the mating spots were resuspended in MB and re-plated onto MB agar plates containing 100 μg/mL erythromycin and grown for at least 48 h at 30°C. Colonies that formed were confirmed to have the pCHP3 plasmid inserted via PCR, with good clones being grown in MB for 24 h at 30°C, shaking at 200 rpm. After incubation, 1 mL of the culture was spun down and washed twice with filtered artificial seawater before being plated on MB agar plates containing 10 mM 4-chloro-L-phenylalanine and incubated at 30°C for at least 72 h. Individual colonies that were then screened for loss of plasmid and confirmed by colony PCR, as well as being extracted and sequenced to confirm the clean knockout. The related primers used for in-frame deletions are listed in Supplementary Table 4.

### Generation and purification of MAC mutants

Mutants in P. luteioviscosa were created using the following protocol and molecular genetics vectors. The regulatory mutant strains were grown in 50 mL artificial seawater with tryptone medium (SWT) at 30°C, 200 rpm overnight in 250 mL flasks. The cells were centrifuged for...
20 min at 4,000 g and 4 °C, with the resulting supernatant being removed. The pellet was gently resuspended in 5 mL cold extraction buffer (20 mM Tris, 1 M NaCl, pH 7.5). The resuspended was then spun down again for 20 min at 4,000 g and 4 °C. The supernatant was carefully pipetted into a new 15 mL conical tube and spun down for 30 min at 7,000 g and 4 °C. After centrifugation, the supernatant was removed and the small pellet was resuspended in residual buffer and stored at 4 °C for use. The resuspended samples were subjected to killing assays and negative-stain EM imaging.

Phylogenetic tree analysis. The phylogenetic trees of different contractile injection systems were examined using the putative sheath proteins on the basis of previous reports. The amino acid sequences were first aligned by the MUSCLE online tool and further subjected to tree reconstruction in the MEGAGA programme. The maximum likelihood method and bootstrap values (1,000 resamples) were applied to assess the robustness of the tree.

Genetic cloning and bacterial intoxication assays. The full-length of the Cgo1 (ALPR1_12695) gene was PCR amplified from the bacterial genome. A His, tag was introduced to the C terminus of the recombinant Cgo1 through the reverse PCR primer. The PCR product was cloned into the pET-Duet (Novagen) vector by the Gibson assembly method or T4 ligation (New England Biolabs). The full-length protein fused with N-terminal periplasmic tag, different point mutations, and truncations of Cgo1 were subsequently generated on the basis of the recombinant Cgo1 construct. The full-length Cgo1 and related mutants were expressed in E. coli BL21 (DE3) cells. In addition, the recombinant Cgo2 (ALPR1_126960) gene, Cgo1/Cgo2 genes, and sGFP gene were also expressed in E. coli BL21 (DE3) growing in the same conditions as that of Cgo1.

To evaluate the intoxication effects of recombinant proteins, the bacteria with the different constructed vectors were grown in LB medium overnight at 37 °C. The overnight bacterial cultures were adjusted to OD600 = 3.0 and serially diluted using LB medium. The 5 μL volumes of serial dilutions were spotted on LB agar containing related antibiotic (100 µg mL⁻¹ ampicillin) and without/with inducer (0.1 mM IPTG). The next day to carrying the empty vector (pET-Duet) and sGFP protein were regarded as negative controls.

Bacterial two-hybrid assays. A bacterial two-hybrid analysis was used to investigate protein–protein interactions following protocols described previously. Briefly, proteins of interest were cloned into one of four bacterial two-hybrid (BACTH) plasmids containing the T8 or T25 subunits of the adenylate cyclase protein. Plasmid fusions were transformed into BTH011 E. coli electroporant cells. The BTH011 cells were grown on LB agar containing ampicillin (100 µg mL⁻¹) and kanamycin (100 µg mL⁻¹) and 1% glucose to suppress expression. Protein–protein interactions were quantified by performing a β-galactosidase assay, with cells being grown overnight at 37 °C and shaking at 200 r.p.m. Protein expression was induced with 1.0 mM IPTG for 8 h at 30 °C. The induced cells were then mixed with a one-step ‘β-gal’ mix. A plate reader was then used to measure the absorbance at 420 nm and 600 nm. The values were then used to calculate Miller Units as previously described.

Killing assays of AlgoCIS against bacteria. To estimate whether AlgoCIS particles could target bacteria, different bacterial strains (E. coli BL21 (DE3) with pET-Duet vector, Vibrio cholerae and Echinolina pacifica) were used in bacterial killing assays with purified wild-type AlgoCIS and Cgo1/Cgo2 samples. Briefly, the different bacterial strains were inoculated into the corresponding medium and grown overnight. The next day, the cultures were adjusted to OD600 = 0.2 by the corresponding medium and then mixed with the equal volume of 0.25 mg mL⁻¹ purified wild-type AlgoCIS or Cgo1/Cgo2, whereas the buffer (20 mM Tris pH7.5, 150 mM NaCl) was regarded as the negative control. The mixtures were further incubated for one additional day on shaker and serial dilutions on agar plates were then performed.

Killing assays against different types of eukaryotic cells. Before treatments, eukaryotic cell strains were grown in multi-well dishes to a confluency of around 150 mM NaCl) was regarded as the negative control. The mixtures were further incubated for one additional day on shaker and serial dilutions on agar plates were then performed.

Before treatments, eukaryotic cell strains were grown in multi-well dishes to a confluency of around 150 mM NaCl) was regarded as the negative control. The mixtures were further incubated for one additional day on shaker and serial dilutions on agar plates were then performed.

The raw numerical data for Figs. 2d and 5a, and Extended Data Figs. 9c and 10d are provided in the source data. Due to the size of the reconstructed tomograms of different AlgoCIS mutants, all tomograms are available from the authors on reasonable request. Source data are provided with this paper.

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Author contributions
J.X. and M.P. conceived the project; J.X. performed the sample preparation, processed the cryoEM data, reconstructed the cryoEM map, built and refined the structural model; C.F.E. developed the bacterial knockout system and performed genetic analyses; C.F.E. performed bacterial two-hybrid assays; Y.-W.L. and J.X. generated bacterial deficient mutants; J.X. and M.F. collected cryoET data and analysed the reconstructed tomograms; J.X. and F.E. performed the sub-tomogram averaging; C.F.E. and J.X. performed killing assay screenings; F.U.N.R. performed assays on choanoflagellates; J.X., C.F.E. and M.P. wrote the manuscript; all authors commented on the manuscript.

Competing interests
C.F.E and M.P. have two provisional patents related to CIS pending in the United States (application no. 62/768,240 and 62/844,988). All other authors declare no competing interests.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Biochemical and EM characterization of AlgoCIS. a: Negative staining EM image of crude sheath preparation from A. machipongonensis showing the typical CIS-like particles in both extended (box) and contracted (circle) states. Bar: 500 nm. The magnified views of the representative particles are shown in the right panels (bars: 50 nm). This experiment was performed ten independent times. b: Negative staining EM images of crude sheath preparations from different A. machipongonensis bacteria (wild-type, AlgoCIS−, and null mutant). Bars: 500 nm. This experiment was performed three independent times. c: Plot showing the number of AlgoCISs per cell as observed by cryoET (averages shown as red lines). AlgoCISs were most abundant at high optical densities (OD600). d: Western blotting analyses of different bacterial samples against the putative inner tube protein (Alg1), showing that the expression level of AlgoCIS is high at high bacterial OD600. The cytosolic protein recA is used as loading control. This experiment was performed three independent times. e: Western blotting analyses of the purified samples from different bacterial culture supernatants. Both putative sheath (Alg2) and inner tube (Alg1) proteins could be detected in the bacterial cultures from wild-type and null mutant, while neither protein could be detected in AlgoCIS− mutant. This experiment was performed three independent times. f: Negative staining EM images of the AlgoCIS purifications from bacterial culture supernatant in different A. machipongonensis bacteria (wild-type, AlgoCIS−, and null mutant). Bars: 500 nm. This experiment was performed three independent times. g: Sub-tomogram average revealing three AlgoCIS structural modules: cap, sheath-tube, and baseplate complex. Corresponding volume slices (perpendicular views) are shown on the right. Bar: 10 nm. h: Central volume slice (left) of sub-tomogram average of AlgoCIS and the corresponding density plot (right) showing that the inner tube lumen has density. i: Self-rotation plot showing that the sub-tomogram averaging of AlgoCIS has 6-fold symmetry features even though 3-fold symmetry was applied in data processing. j: Representative cryoET slice of the purified wild-type AlgoCIS showing the inner tube lumen is filled with density in most particles. The representative filled and empty AlgoCIS particles are highlighted by boxes (filled: cyan; empty: red) and are zoomed in in the right panels. Shown is a 10.8 nm thick slice. Bar: 50 nm. The corresponding density plots of the filled (cyan) and empty (red) AlgoCIS particles are shown in the bottom panel, where the symmetry axis is set as the center. The density measurements were performed independently in three different reconstructed tomograms.
Extended Data Fig. 2 | Workflows for the cryoEM structural determinations of extended AlgoCIS particle and contracted sheath. a: Flowcharts for the cryoEM reconstructions of the different modules of AlgoCIS particle in the extended state, including cap (red), sheath-tube module (white/black), baseplate complex (blue), and the overall AlgoCIS particle. See METHODS and Supplementary Table 3 for details. b: Flowcharts for the cryoEM reconstruction of the contracted AlgoCIS sheath (orange). See METHODS and Supplementary Table 3 for details.
Extended Data Fig. 3 | CryoEM analyses of reconstruction maps and models. a: Gold standard Fourier shell correlation (FSC) curves (left) of the reconstructions of different modules in the extended AlgoCIS particle. The FSC of model vs. map (right) showing the structural qualities of different AlgoCIS modules. b: Local resolution maps of the reconstructions of different modules in the extended AlgoCIS. Bar: 50 Å. c: Gold standard FSC and model vs. map FSC curves of the contracted AlgoCIS sheath. d: Local resolution maps of the contracted AlgoCIS sheath. Bar: 50 Å.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Structural analyses of the cap module and clean in-frame bacterial genetic manipulation in AlgoCIS. a: Shadowed surface and ribbon diagrams of the top view of the hexameric cap protein (orange). Box indicates the position of the central channel of the hexameric cap protein, which is shown in the bottom panel. The side chain of Tyr153 residues (Y153) of the central helix from each subunit points inwards, forming the central channel with a diameter of 10.9 nm. b: Shadowed surface and ribbon diagrams showing the interactions between cap protein and the distal layer of the inner tube. One cap protein (orange) has interactions with three inner tube proteins (Alg1-L22-1: blue; Alg1-L22-2: green; Alg1-L22-3: magenta). The residues of Alg16A participating in contacts are shown with side chains in the bottom panel. c: Ribbon diagrams showing the structures of the cap protein (Alg16A: orange) and the homologous proteins (Afp16: grey; Pvc16: green). The individual domains in homologs are marked with dashed boxes in different colors (NTD: blue; CTD: magenta). The calculated structural R.M.S.D values of Alg1 against NTD domains in the homologs are indicated. The structural superimpositions of Alg16A and homologs are shown on the bottom right. d: Ribbon diagram showing that Alg16B (blue) stands parallel against Alg16A (orange), while the CTD domains of Afp16/Pvc16 (grey dashed line) adopt vertical arrangement against NTD domains. e: Schematic showing detailed information of the vector pCHIP3, which was used for bacterial clean in-frame deletion. f: Schematic showing the workflow of bacterial clean in-frame genetic manipulation in A. machipongonensis using pCHIP3. Please refer to METHODS for details.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Structural analyses of sheath-tube module in extended state. 

**a:** Schematic and ribbon diagrams showing that the sheath protein (Alg2) has four domains. The N- and C-termini are marked with red and green circles. **b:** Structural superpositions of sheath protein (Alg2: orange) with homologous proteins (Pvc4: green; Afp4: grey). The additional domains (domain III and IV), N-, and C-termini of Alg2 are labeled. **c:** Ribbon diagram of a perpendicular slice of the distal sheath layer (Alg2-L23), showing the domain organization of Alg2 in one sheath layer and the interactions between the sheath and inner tube (white). The color code matches panel **a.** Box indicates the interface between sheath and inner tube, which is shown in panel **e.** **d:** Ribbon diagrams showing an extended sheath fragment containing three sheath layers (Ln, Ln+1, and Ln+2) in different colors. The direction of one helical strand (H1) is highlighted by a line. Box indicates the conserved handshake interaction between sheath subunits, which is shown in the right panel. **e:** Shadowed surface and ribbon diagram showing the interactions between the attachment helix of sheath and inner tube. The residues mediating contacts are labeled and shown with side chains. **f:** Structural superpositions of sheath subunits from different layers (distal layer: red; central layers: green; proximal layer: blue) showing the structural diversity in domain IV, N-, and C-termini of Alg2 across different sheath layers. The schematic (right) indicates the position of the different sheath layers on the AlgoCIS particle. **g:** Side view of ribbon diagram showing the overall structure of the cap module and the distal sheath layer. The inner tube proteins, cap, and cap adaptor are colored white. The color code for different domains of one Alg2 subunit (Alg2-L23-H1) matches panel **a,** whereas the neighboring two subunits are shown in different colors (Alg2-L23-H2: cyan, Alg2-L23-H6: brown). **h:** Shadowed surface and ribbon diagrams showing that there are two conformations of domain IV (domain IV-1, 2) in the central sheath layers. The color code for different domains matches panel **a,** while two conformations of domain IV are represented by lines with different colors (domain IV-1: blue; domain IV-2: yellow). **i:** Ribbon diagrams showing the structures of inner tube protein (Alg1: orange) and the homologs (Afp1: grey; Pvc1: green). The structural superpositions of Alg1 with homologs are shown in the bottom right panel. **j:** Surface electrostatic potential of the inner tube showing that the inner surface of tube is dominated by negative charges. Negative and positive electrostatic potentials are colored red and blue, respectively.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Structures of the components of the tube initiator complex and the interactions between plug protein and other baseplate components. a: Ribbon diagrams showing the structures of inner tube layer (Alg1) and two layers in tube initiator complex (Alg5 and Alg7). The color code for one subunit in each layer matches Fig. 1d. Red box indicates the position of the cleaved loop in Alg7, which is shown in panel b. b: Zoom-in of the loop in (a) showing that the distance between two residues (Q168 and P170) is too large to accommodate one amino acid residue (S169), implying that there is a potential cleaved site at this loop. c: Shadowed surface and ribbon diagram showing the structure of plug protein (Alg6), while the corresponding secondary structure and MS analyses is shown in the right panel. The residues are labeled and shown with side chains, with the disordered loop represented by a dashed line. The peptide fragments detected in MS are highlighted yellow and the secondary structural information is shown above the corresponding sequences based on the model. d: Stick and ribbon diagram showing the interactions between Alg6 (yellow), Alg7 (grey), and Alg8 (purple), which is corresponding to the part I in (c). Alg6 is shown in stick style, while other proteins are shown in ribbon style. The key residues mediating contacts are labeled and shown with side chains. e: Stick and ribbon diagrams showing the interactions between Alg6 (yellow) and Alg8 (purple), which is corresponding to the part II-IV in (c). Alg6 is shown in stick style and Alg8 is shown in ribbon style. The key residues mediating contacts are labeled and shown with side chains. f: Negative staining EM images of crude sheath preparation from A. machipongonensis wild-type and the related mutants showing that Alg6 is crucial for particle assembly. No particles were observed in the AlgoCIS ΔAlg6 mutant, which is similar to the AlgoCIS ΔAlg2 mutant. Bars: 500 nm. This experiment was performed three independent times. g: Sequence alignment of Alg6 and putative homolog proteins in MACs (JF50_12690) from P. luteoviolacea and T6SS\(^{14}\) from Ca. Amoebophilus asiaticus (Aasi_1078). Identical residues are shown in white on a red background, while similar residues are shown in red. The blue boxes indicate the conserved positions. The secondary structure of Alg6 is shown above the corresponding sequences. The image is made from Espript\(^{78}\).
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Structural analyses of the AlgoCIS baseplate wedge. a: Ribbon diagram of a perpendicular slice of the AlgoCIS baseplate showing the positions of two different Alg9 protomers (protomer 1: red; protomer 2: green). The other components are colored white. The structural superposition of two Alg9 protomers is shown in the right panel. b: Magnified views of ribbon diagrams showing the interactions between Alg9 (protomer1: red) and other baseplate components (Alg2-L, Alg7, Alg11, and Alg12), which are related to the parts I-III in (a). The color code for different proteins matches Fig. 1d. c: Schematic and ribbon diagrams showing the domain organization of Alg12. The C-terminal part without structural modeling is colored white. The boundary between gp6-like and gp7-like part is marked with dashed line. d: Shadowed surface and ribbon diagrams showing the structural dynamics of the peripheral baseplate in 3D classification analyses. The densities around built-up models are shown transparent, while the dynamic peripheral parts are shown in solid surface. The pair comparisons of dynamic parts between different classes are shown in the right panel. The gp6-like and gp7-like part of Alg12 model is colored blue and red. The color code for different classes matches the 3D classification part in Extended Data Fig. 2a. e: Representative cryoEM image showing the dynamics of tail fibers in the extended AlgoCIS particles. The angles in individual particles were measured and labeled. The lumen of expelled inner tubes (highlighted by black arrowheads) are observed empty upon firing. Bar: 50 nm. f: Ribbon diagram showing the domain organization of Alg11. The color code for different domains matches Fig. 4b. Box indicates the β-sheet assembly of the N-terminal strand and the domain V, which is shown in the right panel. g: Surface electrostatic potentials of the Alg11 domain IV and the overall outer surface of the spike cage revealing that the outer surface of the spike cage is dominated by negative charges, with a positively charged residue (Lys 572) located at the tip. The boundary of one protomer is marked by black contour. Negative and positive electrostatic potentials are colored red and blue, respectively. Bar: 50 Å. h: Ribbon diagrams showing the structures of carbohydrate binding modules (CBM1/2) in Alg11 and the related homologous proteins. Top-left: the overall structure of spike cage. Box I corresponding to part I in the top-left panel: ribbon diagrams showing that the CBM1 might potentially bind to sugar as the homologous protein (PDB entry: 1OF4)39. The surface of sugar is shown in green transparent. The structural superimposition reveals that the residues, participating in the contacts with substrates, are well-conserved in the CBM1. Box II corresponding to part II in the top-left panel: ribbon diagrams showing the structures of CBM2 and the homologous proteins (PDB entries: 4ZX8 and 4JX0)79.
Extended Data Fig. 8 | Conformational changes upon firing reveal the fate of the AlgoCIS cage. a: Side view (left) and bottom view (right) of shadowed surface and ribbon diagrams, showing that the spike cage remains attached to the baseplate and opens. The iris-like ring is intact in contracted AlgoCIS. The color code matches Fig. 4a. The red dashed box indicates the position of one lateral dimer. b: Structural superpositions of Alg11 proteins in the extended and contracted states showing that the conformational change of domain IV mainly contributes to the opening of spike cage. The color code for different domains in the contracted state matches Fig. 4b, while the structure of Alg11 in the extended state is colored white. c: Cutaway view of shadowed surface diagrams of extended (grey) and contracted (yellow) AlgoCIS structures showing that the tail fiber has a large outward tilt upon firing. The extended and contracted maps were both lowpass-filtered to 10 Å. Bar: 50 Å. d: Shadowed surface and ribbon diagram showing that the docking of the T6SS TssK structure [gold, PDB entry: 5MWN] fits the overall structure of the AlgoCIS tail fiber. The tail fiber has a similar shape as the shoulder domain in TssK. e: Cutaway views of shadowed surface and ribbon diagrams of extended (grey) and contracted (yellow) AlgoCIS structures showing that the baseplate core bundles have a higher tilt angle related to the plane of the iris-like ring after contraction. The extended and contracted maps were both lowpass-filtered to 10 Å. Bar: 50 Å. f: Schematic showing the putative contraction and signal transmission process in AlgoCIS upon firing. The color code matches Fig. 1d. The lightning bolts represent the conformational changes of the gp25-like protein Alg9, which triggers sheath contraction.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Characterization and two-hybrid analyses of putative cargo proteins. a: Schematic and disorder probability plot showing the domain organizations and disorder properties of putative cargo protein Cgo1 (ALPR1_12695). The positions of two putative metalloprotease motifs (HExxH) are highlighted by red triangles. The domain organizations of the other putative cargo protein Cgo2 (ALPR1_12690) is shown in the bottom panel. b: Schematic showing the principles of the cross-over methods to generate different cargo protein-deficient mutants. The color code for different genes matches Fig. 1a. c: Two hybrid analyses showing no interaction between the two cargo proteins. Shown are qualitative spot assay on a X-gal plate (top) and the quantification by using a ONPG β-galactosidase assay with fusions to N (KT/18 C) and C-terminal (KNT/18) adenylate cyclase subunits (bottom). Representative experiments are shown. Bars are mean with error bars indicating standard deviation. d: Bacterial spot assays show that the expression of full-length Cgo1 had a killing effect in E. coli (the columns represent the serial dilutions from 10^{-1} to 10^{-6} with a dilution factor 10 per step). Furthermore, the point mutants in putative metalloprotease motifs, or the co-expression with Cgo2, or the fusion with periplasmic tag did not impair the killing effects. The strains carrying pET-Duet/pET-Duet-GFP vectors are negative controls.
Extended Data Fig. 10 | Co-incubation of different types of cells with the purified AlgoCIS or *A. machipongonensis* bacteria. 

**a:** Bacterial killing assays showing that different bacterial strains (*E. coli, V. cholera* and *E. pacifica*) are insensitive to the treatment with AlgoCIS. The purified Cgo1/Cgo2− mutant and buffer are regarded as negative controls.

**b:** Table summarizing different eukaryotic cells that were subjected to the screen against *A. machipongonensis* wild-type and AlgoCIS− bacteria. There was no significant difference in toxicity or lethal effects for all types of cells treated with wild-type/AlgoCIS− bacteria (indicated by ‘N’).

**c:** Insect cell killing assay showing that the insect cells were not sensitive to the treatment with AlgoCIS. The buffer is regarded as negative control. Red (Propidium iodide): dead cells. Green (fluorescence diacetate): live cells. This experiment was performed three independent times with representative images shown. Bars: 100 µm.

**d:** Growth curves of the choanoflagellate *S. rosetta* treated with purified AlgoCIS wild-type and Cgo1/Cgo2− showing that there was no significant effect on the cellular proliferation of the choanoflagellate. The heat denaturation and freeze-and-thaw samples are regarded as negative controls. For the 5 µg/mL treatment, two biologically independent sample timepoints (*n* = 2) were measured with technical replicates (*n* = 3) and for lower concentration treatments (1 µg/mL, 500 ng/mL, 100 ng/mL), one biological sample was measured with technical replicates (*n* = 3). Data are presented as mean values +/- SD of all technical replicates.