Cryo-EM structure of the bacteria-killing type IV secretion system core complex from *Xanthomonas citri*

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Type IV secretion (T4S) systems form the most common and versatile class of secretion systems in bacteria, capable of injecting both proteins and DNAs into host cells. T4S systems are typically composed of 12 components that form 2 major assemblies: the inner membrane complex embedded in the inner membrane and the core complex embedded in both the inner and outer membranes. Here we present the 3.3 Å-resolution cryo-electron microscopy model of the T4S system core complex from *Xanthomonas citri*, a phytopathogen that utilizes this system to kill bacterial competitors. An extensive mutational investigation was performed to probe the vast network of protein–protein interactions in this 1.13-MDa assembly. This structure expands our knowledge of the molecular details of T4S system organization, assembly and evolution.

The Type IV secretion (T4S) systems are ubiquitous nanomachines used by bacteria and archaea to mediate transfer of a large variety of DNAs and proteins into prokaryotic and eukaryotic targets. They mediate bacterial conjugation, and so are essential drivers in the spread of antibiotic resistance genes. In their role as virulence factors in pathogenicity, they secrete transforming DNAs into plants to cause tumorigenic growths and also effector proteins into animal cells to cause widespread infectious diseases. More recently, it was shown that the phytopathogen *Xanthomonas citri* utilizes its T4S system to secrete effectors that are lethal to other bacterial species, thus placing these systems as major fitness determinants of interspecies bacterial competition.

Canonical T4S systems have 12 conserved subunits termed VirB1–11 and VirD4. They can be structurally divided into two subcomplexes, an approximately 2.5-MDa inner membrane (IM) complex and an approximately 1-MDa core complex (Supplementary Fig. 1a). The core complex of canonical T4S systems is composed of 14 copies each of VirB7, VirB9 and VirB10 proteins with most of its mass located within the bacterial periplasm, but also embedded in both the IM and the outer membrane (OM) via amino- and carboxy-terminal transmembrane helices of the VirB10 protein. The core complex architecture consists of two ringed layers, the O and I layers (Supplementary Fig. 1a). The O layer is associated with the OM through a cap that forms a channel composed of 14 two-helix bundles contributed by the C-terminal domain (CTD) of VirB10 (VirB10CTD). This OM channel is butted against just under the OM by a ring formed by VirB10CTD on the inside and the CTD of VirB9 (VirB9CTD) on the outside. In canonical T4S systems, the small (approximately 30–40-residue) VirB7 lipoprotein subunits lie on the outer surface of VirB9 covalently attached to a lipid moiety in the inner leaflet of the OM. Under the O layer lies the I layer formed by the N-terminal domains (NTDs) of VirB9 and VirB10 (VirB9NTD and VirB10NTD, respectively; Supplementary Fig. 1b). To date, the only atomic-resolution information available for T4S systems is the approximately 0.6-MDa structure of the core complex O layer from the conjugative plasmid pKM101 11–13, formed by TraN/VirB7, TraOCTD/VirB9CTD and TraFCTD/VirB10CTD. The atomic-resolution structure of the approximately 0.5-MDa I layer remains unknown.

Here we present the structure of a 1.13 MDa core complex made of the full-length VirB7, VirB9 and VirB10 proteins from *X. citri*, solved by cryo-electron microscopy (cryo-EM) at 3.3 Å resolution. This structure provides the first atomic-resolution model of the I layer as well as that of an O layer that includes an extra VirB7 globular domain that has intriguing similarities to features observed in other pathogenic T4S systems. The roles of specific structural features in the in vivo stabilization of the T4S system and in mediating the killing of bacterial rivals by *X. citri* were investigated.

**Results**

**General architecture of the X. citri T4S system core complex.**

The *X. citri* VirB7, VirB9 and VirB10 proteins were overexpressed and purified from *Escherichia coli* (Fig. 1a). A cryo-EM data set of approximately 185,000 particles (Fig. 1b, Supplementary Table 1) was used to compute an electron density map with an average resolution of 3.3 Å and a local resolution extending to 3.1 Å (Fig. 1c,d and Supplementary Fig. 1c–e). Top/bottom-view class averages clearly indicated an overall 14-fold symmetry (Fig. 1c). This 1.13 MDa core complex resembles a flying saucer decorated

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The compact core region, including the rest of VirB7, forms the O layer of the core complex while the VirB9_{NTD} domains and the mostly disordered VirB10_{NTD} domains form the I layer (Fig. 2c).

The X. citri O layer. The X. citri O layer (minus the VirB7 N0 domains) is homologous to that of pKM101 \(1^5\) (overall root-mean-square deviation of 2.6 Å; Fig. 3c and Supplementary Fig. 3), as is the nature of most of the intermolecular contacts and the contact surface areas between the 3 proteins in this region (reported in Supplementary Table 2 for X. citri). A detailed comparison of the O layers in these two structures is presented in Supplementary Fig. 3.

An important feature of the X. citri core complex O layer is the globular C-terminal N0 domain \(1^6\) of VirB7 (residues 54–132) composed of two central α-helices flanked on one side by a three-stranded β-sheet and on the other by a two-stranded β-sheet and a small 3_10 helix (Figs. 3 and 4c). Seen from above, these N0 domains wrap clockwise around the outer perimeter of the O layer.

Fig. 1 | Biochemistry and EM map and model of the X. citri T4S system core complex. a, SDS-PAGE of the VirB7-VirB9-VirB10 core complex. Left lane labelled ‘Marker’: molecular weight markers, with the molecular weight for each band shown on the left. Right lane labelled ‘CC’: purified core complex, with the three components labelled on the right. Purification assays were repeated more than ten times with similar results (see also Supplementary Fig. 9a). b, EM micrograph of the X. citri core complex, with some particles highlighted in blue circles. Scale bar, 50 nm. Experiments were repeated at least eight times with similar results. c, Representative top-, tilt- and side-view 2D class averages obtained using RELION 2.0 (see Methods for more information). d, Overview of the 3.3 Å electron density, contoured at the 4σ level. An electron density map contoured at the 4σ level is shown in a chicken wire representation, colour-coded in grey-blue. The final model built into the map is shown in stick representation colour-coded dark blue, red, yellow and light blue for nitrogen, oxygen, sulfur and carbon atoms, respectively. Secondary structures are labelled. The regions depicted are both from the VirB10_CTD (left panel for α-helices and right panel for β-strands). f, Topology secondary-structure diagrams of VirB7 (red), VirB9 (green) and VirB10 (blue). β-strands and α-helices are represented as arrows and cylinders, respectively. Regions for which no electron density was observed are indicated by dashed lines. Note that the 182 N-terminal residues of VirB10 were disordered and could not be traced except for a small helix corresponding to residues 150–161. This is not unexpected considering that the 101-amino-acid stretch (residues 84–184) between the IM-spanning helix and the globular VirB10_{CTD} is rich in proline residues (Supplementary Fig. 2).
Fig. 2 | Structure of the X. citri T4S system core complex. a, Top view of the structure in a ribbon representation with one VirB7–VirB9–VirB10 heterotrimer shown in red, green and blue, respectively (colouring maintained in b–e). The heterotrimer numbering used in this study is indicated, with the coloured heterotrimer serving as a reference and numbered 0. b, c, Top view (b) and side view (c, top) of the structure in surface representation. c, Bottom, cut-away side view of the model. External and internal dimensions of specific structural features are indicated in b and c. The 80 Å opening at the bottom of the I chamber is similar to that observed for the 8.5 Å cryo-EM structure of the elastase-digested pKM101 core complex17. d, e, Side view of the structure in surface representation as in c but rotated 60° anticlockwise to show heterotrimer 0 more clearly. e, Zoomed-in view of the region delimited by the dashed line in d.

The atomic structure of the I layer. Another significant feature presented here is the atomic-resolution structure of the I layer. Most of the observed density in this region is derived from the 14 VirB9NTD domains, each of which exhibits a β-sandwich fold made of five-stranded and four-stranded β-sheets, with one α-helix (α1) lying under the β-sandwich in a loop between β-strands 8 and 9 (Figs. 3b and 4d). VirB9NTD domains interact extensively with each other (Fig. 4a–d and Supplementary Fig. 4d) to form the I-layer ring with an internal diameter of 80 Å at its base (Fig. 2c). The VirB9NTD–VirB9NTD interface (720 Å²) (Supplementary Table 2) arises from specific contacts between the O and I layers.

The VirB9NTD is expected to interact with at least part of the VirB10NTD in the I layer17, although specific details regarding this interaction are not yet available. While most of the VirB10NTD seems to be disordered in the present structure, we observed some significant electron density at the base of the I layer between adjacent VirB9NTD domains (Fig. 4f). This patch of isolated electron density was of sufficiently high resolution that it could be traced and built as a 12-residue α-helix. To test whether this density might correspond to a structured region of the VirB10NTD, we expressed and purified 13C/15N-labelled VirB10NTD (residues 85–182) and unlabelled VirB9NTD (residues 27–150) for NMR-based interaction studies, as described in detail in Supplementary Fig. 5 and Supplementary Table 3. These NMR studies, in conjunction with the side-chain pattern of residues in the electron density map, led us to assign the density of the α-helix in the I-layer region to the sequence P150TLLERRILAE161 of VirB10NTD (Fig. 4f). Hereafter, this helix will be referred to as the VirB10NTD150–161 helix.

Disordered VirB9 and VirB10 linkers and absence of stereospecific contacts between the O and I layers. Long linkers between domains in all three proteins project part of their structure away from the heterotrimERIC core region in the O layer (Fig. 2). Of particular interest are the linkers that join the NTD and CTD domains of the VirB9 and VirB10 subunits between the I and O layers, respectively. In the case of VirB9, a long linker (residues 131–161) emerges from the VirB9NTD β-sandwich and meanders to the VirB9CTD ~37 Å away in a clockwise (+) direction (Fig. 3a,b). This linker is partially
disordered, with a 15 Å stretch corresponding to residues 142–147 that could not be traced, but has one notable structural feature, α-helix 2 (α2), that is involved in numerous interactions with adjacent and non-adjacent heterotrimers in the O layer (specifically VirB10NTD, of heterotrimers –1 and –2; Fig. 4e and Supplementary Fig. 4e) but very few, if any, contacts within the I layer.

In the case of VirB10, the first secondary-structure element in the O layer, β1 (residues 185–191), extends in an anticlockwise (−) direction and inserts itself between the β-strands β11 and β17 at the base of the β-barrel of VirB10CTD, in the adjacent heterotrimer (Fig. 4b and Supplementary Fig. 4b). This interaction is similar to that observed in the pKM101 O layer for the β-strand in the so-called ‘lever arm’ that precedes the TraFCTD/VirB10CTD helix in the pKM101 O-layer structure15. This puts restrictions on the secondary structure and direction to be adopted by the intervening 21-residue sequence between the VirB10NTD,150–161 helix and VirB10CTD (Supplementary Fig. 6a).

Fig. 3 | The heterotrimer of the X. citri T4S system core complex structure and comparison with that of pKM101 from E. coli. The X. citri VirB7–VirB9–VirB10 heterotrimer. a, Proteins are shown in a ribbon representation and are colour-coded as in Fig. 2. Specific domains are labelled, as are the secondary structures in each protein. The disordered linkers between VirB9 NTD and CTD, between the two α-helices of the VirB10 ‘antennae’ and between the CTD of VirB10 and VirB10NTD,150–161 helix are represented by dashed lines. The orientation is as in Fig. 2a, b, The same as in a, but rotated 90° to correspond to the side view shown in Fig. 2c. The box delimits the central compact core. c, Superposition of the structure of the X. citri heterotrimer (in red, green and blue) with that of the pKM101 complex (grey) composed of TraN/VirB7, TraCCTD/VirB9CTD and TraFCTD/VirB10CTD.

Functional analysis of structural features of the Xanthomonas core complex. In vivo assays of T4S system assembly and function. Figure 5a (left panel) shows the time course of a colorimetric bacterial killing assay in which the rate of chlorophenol red-β-β-d-galactopyranoside (CPRG) cleavage is proportional to the number of E. coli cells that have been lysed by X. citri in a T4S system-dependent manner (see Methods for details). Interestingly, X. citri virB10–msf-GFP cells, in which the virB10 gene was substituted with a chimaeric gene that codes for VirB10 and monomeric superfolder green fluorescent protein (msfGFP) fused to its Cterminus, are capable of killing E. coli cells in these competition assays with an efficiency approximately 81% that of wild-type X. citri cells (Figs. 5a and 6a and Supplementary Table 4). Furthermore, microscopy analysis shows that these X. citri virB10–msfGFP cells have distinct fluorescent foci in the bacterial periplasm (Figs. 5a and 6a and Supplementary Table 4). On the other hand, X. citri virB10–msfGFP cells in which
the virB7 gene was deleted are not able to kill *E. coli* and do not present periplasmic foci (Fig. 5b). We therefore decided to use this *X. citri* virB10–msfGFP strain as a reference in experiments designed to test the effect of mutations on the assembly of a stable core complex in the *X. citri* periplasm. Several experimental controls that were performed to validate the use of the *X. citri* virB10–msfGFP strain are described in detail in Supplementary Note and Supplementary Fig. 7. Thirty-four specific point mutations in the chromosomal copies of the *virB7*, *virB9* or *virB10* genes were produced in one or both of two different genetic backgrounds: wild-type *X. citri* (27 of 34 mutations) and/or the *X. citri* virB10–msfGFP strain (33 of 34 mutations) (Supplementary Table 4). Figure 5b presents the fluorescence images and real-time killing assay curves of several representative mutants and the data obtained for all of the mutants (in both backgrounds) are summarized in Fig. 6, Supplementary Table 4 and Supplementary Fig. 7. The fluorescence microscopy images of hundreds to thousands of cells from each strain were analysed to obtain a distribution of the number of fluorescent foci per cell (Fig. 6a and Supplementary Table 4). Below, we describe the phenotypes of these mutants in the context of their periplasmic stability of specific structural features of the *X. citri* core complex.

**VirB7–VirB9 and VirB7–VirB7 interactions.** Three VirB7 residues, Asn 38, Val 37 and Trp 34, shown here to interact with VirB9 in the same heterotrimer (Supplementary Fig. 4a) and for which mutations were shown previously to reduce the strength of the VirB9–VirB7 interaction with increasing degrees of severity in vitro (N38A < V37G < W34A) also presented the same order in severity of loss of T4S system function in the *E. coli* lysis assay (Figs. 5 and 6 and Supplementary Table 4). Deletion of the whole N0 domain (by introducing a stop codon at position 39 of the virB7 gene) abolished T4S system-dependent killing in both
Fig. 5 | Effect of specific mutations in the core complex on T4S system-mediated cell lysis of neighbouring E. coli cells and VirB10 localization. a, Left, bacterial killing assay measuring the ability of X. citri to use the T4S system to lyse neighbouring E. coli cells. The results obtained for wild-type X. citri cells (dashed grey line) are compared to the assay performed using the X. citri virB10–msfGFP strain (solid black line). The dashed black horizontal line corresponds to the no lysis baseline after subtracting the E. coli-only background signal from the data. Data are mean ± s.d. (n = 9 for X. citri virB10–msfGFP and n = 27 for wild-type X. citri). Middle, representative image of the X. citri virB10–msfGFP strain obtained by epifluorescence microscopy displaying discrete msfGFP foci that indicate the presence of T4S systems. The image shows the msfGFP intensity levels of a 0.5-µm region containing the focal plane of the cells. The inset shows the superposition of the locations of fluorescent VirB10–msfGFP foci obtained from 100 individual X. citri virB10–msfGFP cells. Right, an enhanced image obtained by deconvolution of the obtained z-slices (not used for quantification) more clearly showing discrete foci (see Methods). Scale bars, 5 µm. The X. citri virB10–msfGFP strain was imaged and analysed at least six times independently with similar results.

b, Representative epifluorescence microscopy images (as described in a) for a selected series of X. citri virB10–msfGFP mutant strains in VirB7, VirB9 or VirB10. Note that ΔVirB7 and VirB10C206S cells are mostly devoid of fluorescence. Other strains, such as VirB7W34A, present more diffuse fluorescence and lack clear foci. Also note that the few foci shown in the cell contour insets of mutants severely deficient in killing (see below) are due to occasional background detection. Scale bars, 5 µm. All X. citri virB10–msfGFP mutant strains were imaged and analysed together on two separate occasions independently with similar results.

c, Bacterial killing assays of the X. citri virB10–msfGFP strains shown in b. Each X. citri virB10–msfGFP mutant was compared to the X. citri virB10–msfGFP strain (solid black line). Data are mean ± s.d. (n = 9 for X. citri virB10–msfGFP and n = 4 for each mutant).
Fig. 6 | Killing efficiency is correlated with T4S system assembly in \textit{X. citri}. \textbf{a}, Top, the relative number of VirB10-msfGFP foci per cell plotted for each strain. The distribution of cells according to the number of foci per cell is represented by the randomly placed shaded circles in each bin. Tukey box-and-whisker plots depict the data: black central line (median), box (first and third quartiles), whiskers (data within 1.5 interquartile range), green triangles (mean) (see Supplementary Table 4). The sample size (\(n\)) of cells of each strain analysed by fluorescence microscopy (from two independent experiments) is listed at the top. NA, not available. Bottom, the mean (\(\pm\) s.d.) relative capacity of each mutant to lyse \textit{E. coli} cells in a \textit{X. citri}/\textit{E. coli} co-culture (as in Fig. 5 and Supplementary Table 4). The red dots represent mutants produced in the VirB10-msfGFP background and the black dots represent mutants produced in the non-GFP background. In competitions using GFP background strains, \(n\) = 4 (except for VirB9V29D-I103D (\(n\) = 3), VirB10T325-T335 (\(n\) = 3), \(\Delta\)VirB7 (\(n\) = 6) and VirB10-msfGFP (\(n\) = 9)). In competitions using non-GFP background strains, \(n\) = 3. \textbf{b}, Western blot assays using polyclonal antibodies (Ab) against specific T4S system components or against msfGFP in different \textit{X. citri} virB10-msfGFP mutant strains (see Methods). The first lane contains total protein extract from \textit{E. coli} BL21(DE3) expressing the \textit{X. citri} core complex with normal length VirB10 as does that for the VirB9A142-S147 mutant (red asterisks), which was obtained only in the non-GFP genomic background. Experiments were repeated three times with similar results. The dagger indicates mutations in the VirB9 linker and the double dagger indicates mutations in the VirB10 linker for VirB10Q175-D182, or the VirB10 antenna for VirB10T325-T335. The coloured diamonds denote the values of the relative killing efficiency according to the code: less than 0.3 (red), 0.3 to 0.7 (yellow), greater than 0.7 (green) (see \textbf{a} and Supplementary Table 4). WT, wild type. Theoretical molecular weight (in kDa) for each mature protein is shown at the right. \textbf{c}, Western blot detection of VirB10 in mutant strains produced in the non-GFP genomic background. Annotations are the same as in \textbf{b}. Experiments were repeated twice with similar results. Full western blots are presented in Supplementary Fig. 9b–g.
VirB10CTD–VirB10CTD and VirB9CTD–VirB10CTD Interactions in the O layer. The large interface between VirB10CTD domains has a notably high number of hydrophilic side chains between neighboring β-barrels. For example, a cluster of acidic and basic residues forms an intricate network of salt bridges: Arg200 and Asp380 from one subunit and Asp263, Arg264 and Asp272 from the subunit in heterotrimer +1 (Supplementary Fig. 4b). Disruption of this network of ionic interactions by introducing substitutions in VirB10 residues Arg264 or Asp380 drastically reduces T4S system-mediated bacterial killing efficiency in both non-GFP and VirB10–msfGFP backgrounds with a concomitant total absence of fluorescent foci (Fig. 6a and Supplementary Table 4). Another set of salt bridges is made between VirB10 residues Arg211 and Asp281 and VirB9CTD Glu216 from the preceding heterotrimer (Supplementary Fig. 6b). Disruption of these interactions by mutating Arg211 to glutamate in the VirB10–msfGFP background only moderately reduces killing activity and the mean number of foci (Supplementary Table 4). Finally, we probed another three-way interaction involving the intramolecular salt bridge formed by residues Arg205 and Glu226 in the VirB10CTD, which also H-bond to the side chains or backbone carbonyl groups from residues Thr201, Tyr235 and Leu219 in VirB9CTD domains from the same heterotrimer and heterotrimer −1 (Supplementary Fig. 6b). Mutating VirB10 residue Arg205 to alanine significantly reduces (but does not abolish) foci formation and abolishes killing in the VirB10–msfGFP background but, surprisingly, has no effect on bacterial killing efficiency in the non-GFP background. The reason for this discrepancy may be related to the fact that Arg205 is located in β3 that pairs with β16 just before the VirB10 C terminus (Fig. 1f). Mutation of VirB10 Glu226 in the VirB10–msfGFP background had a more moderate effect while the double R205A/E226A mutant abolishes foci formation and killing (Fig. 6a and Supplementary Table 4). Taken together, these results suggest that ionic interactions at the VirB10CTD–VirB10CTD interface are more important for T4S stability and function than those encountered at the VirB10CTD–VirB9CTD interface.

Mutation of Cys206 in the well-conserved intramolecular disulfide bond with Cys222 in the VirB10CTD (Supplementary Fig. 4b) results in abolition of T4S system function in both VirB10–msfGFP and non-GFP backgrounds (Fig. 6a and Supplementary Table 4). This mutation seems to be particularly disruptive since microcosmic analysis of X. citri virB10C206S–msfGFP cells presented no periplasmic foci and very little background fluorescence (Fig. 5b, Supplementary Table 4 and Supplementary Fig. 7). In fact, the only other VirB9 or VirB10 mutants to present significantly reduced periplasmic fluorescence are also mutations in the VirB10CTD (R264D, D380A, R264D/D380R and R205A/E226A discussed above; Supplementary Table 4 and Supplementary Fig. 7).

The pores in the outer membrane formed by the VirB10 antennae. In both X. citri and pKM101 complex structures, the 14 VirB10 α1/α2 helix pairs come together to form a diaphragm-like
pore in the outer membrane (Fig. 4а and Supplementary Fig. 3). In the X. citri structure, the cryo-EM map shows no density for VirB10 residues 324–337 (sequence: TTTIVGSGIVTQQ; Supplementary Fig. 2c) in the extracellular loop between α1 and α2. Deletion of a major portion of this loop (residues 325–335) in the VirB10–msf-GFP background produced only a moderate reduction in foci formation and killing efficiency (Fig. 6а and Supplementary Table 4). This implies that either the core complex can undergo a major conformational change to further close the pore or the pore is naturally gated by other components of the T4S system (for example, VirB2 or VirB5). We note that similar deletions in the E. coli pKM101 Tra and Agrobacterium tumefaciens VirB/VirD4 systems retained partial function in DNA transfer assays..

Discussion

This 3.3 Å-resolution structure of the X. citri T4S system core complex provides the first atomic-resolution model of an I layer containing VirB9NTD domains and their specific interactions with a helical region of the VirB10NTD as well as the detailed structure of an expanded O layer containing VirB7 N0 domains. Mutations that interfere with the extensive, highly stereospecific interfaces that maintain the O and I layers intact, characterized by deeply buried hydrophobic residues and intricate networks of ionic interactions, produced strong defects in T4S system assembly and function, as did mutations that interfered in the less extensive VirB9NTD–VirB10NTD–VirB10NTD–VirB10NTD interactions of one ring with respect to the other in terms of rotation and/or dissociation at different stages of the substrate translocation process.

The disposition of VirB7 N0 domains on the outer periphery of the O layer provides a detailed view of functionally important and intriguing structural variability within the canonical group of T4S systems. There is, in fact, a large amount of sequence and size diversity in proteins annotated as VirB7 in the protein sequence databases, but their C-terminal extensions have no apparent sequence similarity to the N0 domain sequences found in Xanthomonadaceae VirB7 proteins. We have previously pointed out the topological similarities between X. citri VirB7 and Legionella pneumophila DotD proteins. Interestingly, we were able to find a low amount of sequence similarity between X. citri VirB7 and the N-terminal half of the Helicobacter pylori CagT protein and, as described in Supplementary Discussion and Supplementary Fig. 8, the disposition of VirB7 N0 domains on the peripheral surface of the X. citri core complex may be analogous, if not homologous, to the spokes that decorate the inner ring of the H. pylori Cag T4S system.

The clear correlation between the number of fluorescent foci and the killing activity of the mutants in the VirB10–msfGFP background (Fig. 6а) was surprising since we had expected to isolate at least a few mutants that were able to assemble (that is, present near wild-type levels of foci) but were non-functional. In hindsight, this may be because the mutants were designed to disrupt subunit–subunit interactions. Future studies could test other mutants in the channel lumen expected not to affect the core complex stability but rather perturb weaker or transient interactions with other T4S system components or with substrates during translocation.

Methods

Cloning, expression and purification of the T4S system core complex of X. citri. The sequence coding for the virB7, virB8, virB9 and virB10 genes in the X. citri genome was amplified by PCR as a single fragment using oligonucleotides containing NdeI and NotI restriction sites (Supplementary Table 5). The PCR fragment was then introduced into the pET-24a-based pGGS2a vector (designed to add a C-terminal Strep-tag; see Supplementary Table 5 for primers used to produce the pGGS2a vector) cut by the same enzymes to yield the plasmid pGGS2a-virB7-virB10_strep, which was used to transform the BL21(DE3) strain. Protein expression was induced by addition of 0.25 mM IPTG during the exponential growth phase for 18 h at 18°C. After this time, cells were collected by centrifugation at 8,000 g for 20 min. Crude membrane fraction was isolated by ultracentrifugation (120,000 g for 1 h) after cells were broken using an EmulsiFlex-C5 system (Avantium) and a low-speed centrifugation step (15,000 g for 30 min). Membrane proteins were then solubilized overnight with mild agitation in 50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 0.8% LDAO (Anatrace) and 1% DDM (Anatrace), and insoluble material was removed by centrifugation (100,000 g for 40 min). The detergent-solubilized protein fraction was loaded onto a Streptactin Sepharose affinity column (GE Healthcare). The column was washed with 50 mM Tris-HCl (pH 8.0), 150 mM NaCl and 10 mM LDAO and eluted in 200 mM NaCl buffer supplemented with 10 mM 4-mercaptobutylamine (Sigma-Aldrich). The PCR eluted proteins were then concentrated by filtration using a 100-kDa cutoff spin column and loaded onto a HiLoad Superose 6 GL 16/100 gel filtration column (GE Healthcare) equilibrated in 20 mM Tris-HCl (pH 8.0), 200 mM NaCl and 10 mM LDAO. Protein content was analysed by SDS-PAGE and Coomassie blue staining and concentration was determined using the bichinchoninic acid (BCA) method (Sigma-Aldrich).

Expression and purification of VirB9-ntd and VirB10-ntd. Recombinant X. citri VirB9-ntd was expressed in E. coli BL21(DE3) RP cells harbouring the plasmid pET28a-virB9A-2xHis-2xHis, in which the virB9-ntd (residues 27–130) coding sequence was cloned between the NdeI and BamHI restriction sites (Supplementary Table 5). Expression of VirB9-ntd was induced in a culture (OD600 = 0.8) grown in 2xTY medium at 25°C by the addition of 0.4 mM IPTG. After 8 h, cells were collected by centrifugation, resuspended in 20 mM Tris-HCl (pH 8.0), 200 mM NaCl and 20 mM imidazole, and lysed using a French press. The lysate was clarified by centrifugation at 27,000 g for 40 min. The supernatant was applied onto a Ni2+-HiTrap column (GE Healthcare) equilibrated with 20 mM Tris-HCl (pH 8.0), 200 mM NaCl and 20 mM imidazole and eluted using a linear 20–500 mM imidazole gradient over 20 column volumes. Fractions containing VirB9-ntd were pooled and applied onto a HiLoad Superdex 75 26/600 column (GE Healthcare) equilibrated with 20 mM Tris-HCl (pH 8.0) and 100 mM NaCl. After size-exclusion separation, buffer exchange to 20 mM sodium acetate (pH 5.5) and 200 mM NaCl was performed during concentration to 270 μM using a spin concentrator.

The DNA sequence encoding VirB10-ntd (residues 85–182) was chemically synthesized with codon optimization for expression in E. coli and cloned into the expression vector pET28a between the NdeI and BamHI restriction sites of the plasmid pET28a-virB10A-2xHis-2xHis, which was transformed into E. coli BL21(DE3) RP cells in M9 minimal medium containing 0.5 mM IPTG when the bacterial culture reached OD600 = 0.4. Cells were harvested, resuspended in 20 mM Tris-HCl (pH 8.0) and 200 mM NaCl, and lysed using a French press. The lysate was centrifuged at 27,000 g for 40 min and the supernatant was loaded onto a Ni2+-HiTrap column (GE Healthcare) equilibrated with 20 mM Tris-HCl (pH 8.0), 200 mM NaCl and 20 mM imidazole and eluted using an imidazole gradient as described above. Fractions containing His–VirB10-ntd were combined and dialysed against 150 mM NaCl overnight at 4°C. His-tag was removed by incubation with thrombin–agarose resin (Sigma-Aldrich) for 5 h at 25°C and subsequent size-exclusion separation and buffer exchange to 20 mM sodium acetate (pH 5.5), 150 mM NaCl was performed during concentration to 270 μM using a spin concentrator.

EM sample preparation and data collection. To evaluate the quality and homogeneity of the X. citri core complex purifications, aliquots (~3 μl) were applied on glow-discharged, carbon-coated copper grids (Agar Scientific). After incubation of the sample for ~2 min at room temperature, the grids were rapidly washed with three successive drops of deionized water and then exposed to a solution of 2% uranyl acetate. Images were recorded on either a JEM 2100
microscope (operating at 200 kV) or a Philips Tecnai T12 microscope (operating at 120 kV), both using a LaB6 filament, and coupled to a CCD (charge-coupled device). For cryo-EM sample preparation, aliquots (~4 μl) of purified X. citri core complex at a concentration of approximately 0.3 mg ml−1 were deposited onto glow-discharged ultrathin carbon film on lacey 400 mesh copper grids (Agar Scientific). A Vitrobot Mark IV (FEI) plunge-freezing device operating at 22°C and 100% humidity was used to incubate the sample with the grid for 30 s and blot for 4.5 s before vitrification in liquid ethane. The data were collected at the EBC facility at the Diamond synchrotron using an FEI Titan Krios transmission electron microscope, operated at 300 kV and equipped with an energy filter (Gatan GIF Quantum). Data sets (1,469 micrographs) were automatically acquired with EPU software (FEI), recorded on a K2 Summit direct electron detector (Gatan) operated in super-resolution counting mode with a physical pixel size of 1.06 Å per pixel (0.53 Å per super-resolution pixel). The detector was placed at the end of a GIF Quantum energy filter optics to deliver energy-down low-loss λ/2m electrons. The K2 Summit detector was equipped with a cryocooler (Gatan 626-103) and operated at 80 K at a voltage of 70 kV. The total exposure time was 12 s and intermediate frames were recorded every 0.3 s giving an accumulated dose of ~60 e− A−2 and a total of 40 frames per image. The defocus range used for data collection was from approximately −1.0 μm to approximately −3.0 μm.

Cryo-EM image processing and reconstruction. All micrographs were first subject to patch-based motion correction using MotionCor219, with twofold binning (1.06 Å per pixel after binning) and dose-weighting. The contrast transfer functions of the camera were estimated from the micrographs or estimated from a reference-free 2D class average of all the 40 images. Approximately 4,000 particles were manually picked from selected micrographs to generate representative two-dimensional (2D) class averages. These classes were used as templates for automated particle picking for the entire data set. A total of 185,000 particles from set 1 and 19,150 from set 2 were used for all post-EMNMR processing steps, a solvent mask and a B-factor of 350 Å2 were deposited onto glow-discharged carbon film. For cryo-EM sample preparation, aliquots (~4 μl) of the 10 mM IPTG, washed once and concentrated 10 times. Five microlitres of these cultures were plated onto LB-agar plates supplemented with sucrose (10 g l−1 tryptone, 5 g l−1 yeast extract, 60 g l−1 sucrose and 15 g l−1 agar), selecting for cells that recently excised the integrated pNPTS138 plasmid by a two-homologous recombination between the duplicated 1,000 bp upstream or downstream of the tolC or pnp Shine-Dalgarno sequences. The pNPTS138 vector was digested and cloned into pBluescript SK− using EcoRI and HindIII, the linearized vector was electro-transformed into E. coli and selected on LB−agar plates containing KmR.

Construction of X. citri virB10–msfGFP strain. The msfGFP gene was amplified from pDL1029p, introducing a flexible N-terminal linker (SSGGG). Separately, an in-frame X. citri tolC upstream and downstream regions from the tolC stop codon of virB10 were amplified by PCR (Phusion polymerase, Thermo Scientific) from X. citri strain 306 genomic DNA, using primers containing either homology to the pNPTS138 vector (KmR, sacB, ColE1 ori) or the msfGFP gene (Supplementary Table 5). The three fragments were cloned into the pNPTS138 vector using Gibson Assembly and transformed into the desired or wild-type DNA version by PCR, using primers annealing outside the cloned region and confirmed by Sanger sequencing of the PCR product.

Construction of X. citri mutant strains. Due to the high number of mutant strains to be tested in the components of the core complex of X. citri, we decided on the basis of their viability in the genome. For VirB7, all mutations (W34A, V37G, N38A, F40A, I47A/P48A/L49A, ΔN0 and M60A) were included in one single group; for VirB9, mutations V29D, I103D, Y126D, F67R, Y81A, Y181E and W113A were included in group A, mutations S137A, F138A, ΔA142–S144, ΔP146 and ΔN147 were included in group B, and mutations R205A/E226A were included in group C. Thus, for each group, there was a pNPTS138-based plasmid containing ~1,000 bp upstream and downstream of the nucleotide located at the centre of the respective group, by PCR using specific primers for each case (In-Fusion HD Cloning System, Clontech, Supplementary Table 5) and genomic DNA from the X. citri strain 306 (wild-type) or X. citri virB10–msfGFP strain, as previously described. All of the mutations (single, double, triple and deletions) were introduced into the corresponding gNPTS138-derived vector by PCR and sesigation using specific primers for each case (In-Fusion HD Cloning System, Clontech, Supplementary Table 5). Note, that in some cases (combinations of mutations V29D, I103D, Y126D and F67R/D88R) no particles were found, as these particles are only detectable by extracellular LacZ. As such, the rate of CRPG cleavage is proportional to the number of E. coli cells that have lysed (X. citri strain 306 lacks an active lacZ gene). To each well of a U-shaped bottom 96-well plate (Greiner), 100 μl of a mixture of 0.5x buffer A (0.1% (NH4)2SO4, 0.3% Na2HPO4, 0.15% KH2PO4, 0.15% NaCl), 1.5% agarose and 40 μg/ml CRPG (Sigma-Aldrich) was added, and plates were thoroughly dried under a laminar flow. X. citri cells were grown overnight in 96-well plates in AB medium (0.2% (NH4)2SO4, 0.6% Na2HPO4, 0.3% KH2PO4, 0.3% NaCl, 0.1 mM CaCl2, 1 mM MgCl2, 3 μM FeCl3) supplemented with 0.2% sucrose, 0.2% casamino acids, 10 μg ml−1 thiamine and 25 μg ml−1 uracil. After determination of the optical density of the culture of each X. citri strain using a TECAN plate reader between 600.5 and 650 nm, 10 μl of an E. coli GM1655 culture that had been grown to OD600=1 in the presence of 0.2 mM IPTG, washed once and concentrated 10 times. Five microfilters of these mixtures were immediately added to the 96-well plate without puncturing or damaging the agarose, covered with a transparent seal and quickly thereafter absorbance at 572 nm (Abs572) was monitored in a 96-well plate reader for at least 200 min (SpectraMax Paradigm, Molecular Devices). Note, to yield consistent results for this assay, plates must be very dry so that the 5 μl mixtures are absorbed within minutes. Mutants were organized in 96-well plates together with wild-type X. citri, X. citri virB10–msfGFP and E. coli controls. Assays were performed 3 or 4 times for each mutant, 9 times for X. citri virB10–msfGFP and 27 times for wild-type X. citri, and the averages of the resulting killing curves are presented with their standard deviations. To control for putative secondary modifications in the mutant’s genetic background beyond the targeted loci, wild-type revertant strains obtained during the selection of each mutant were all confirmed to have wild-type killing efficiencies. The A600 values were processed using RSstudio software27 and plotted using the ggplot2 package28. Background intensities obtained from the mean
of $A_{\text{obs}}$ values of two repeats containing only $E. coli$ cells were subtracted from the data series. Hereafter, each series was normalized to the initial $A_{\text{obs}}$ values from each $X. citri$ culture to correct for small differences in $OD_{\text{meas}}$ after overnight growth (note that these small differences could be in growth for the mutants, except for mutant VirB10 (G755V452, which also showed smaller cell sizes)). Next, the initial $A_{\text{obs}}$ value at time 0 min was subtracted from all subsequent time points to correct for small differences in initial $A_{\text{obs}}$ measurements. Finally, the data for each set of mutants (virB10–msfGFP and non-fluorescent backgrounds) was normalized to their wild-type counterparts, leading to the final figures shown with $A_{\text{obs}} = 0$ serving as the $E. coli$ control baseline. Furthermore, to produce a single numerical comparison of the data, the mean slopes (obtained by calculating the discrete first derivative) between time point 50 and 100 min of the obtained normalized $A_{\text{obs}}$ versus time curves of mutant and wild-type cells were compared to obtain the fraction of killing efficiency of each mutant compared to the wild-type $X. citri$ strain (Supplementary Table 4). These calculations did not in any way distort the conclusions initially drawn based on the analysis of the raw data.

Fluorescence microscopy image acquisition and analysis. To analyse the distribution of msfGFP-labelled VirB10 in the $X. citri$ periplasm with mutant and wild-type virB10 loci, cells were grown in the same conditions as for the CPRG-competition assays described above. In brief, 1 µl of cell suspension was spotted on a thin agarose slab containing 1x buffer A (0.2% (NH4)2SO4, 0.6% Na2HPO4, 0.3% KH2PO4, 0.5% NaCl)2 and 2% agarose and covered with a no. 1.5 cover glass (Corning). Plasmid and msfGFP expression were monitored using a Leica DMI-8 epifluorescence microscope. msfGFP emissions were captured using a 1,500 ms exposure time at maximum excitation light intensities. The microscope was equipped with a DFC355 FX camera (Leica), a HC PL APO 100×/1.4 oil ph3 objective (Leica) and a GFP excitation–emission band-pass filter cube (Ex.: 470/40, 510–550 nm; Em.: 525/50; Leica). To increase spatial resolution, 100-0.5-µm Z-planes were stacked from a 0.5-µm step and 10% of the frames were obtained from the median plane of focus. This allowed for a better signal-to-noise ratio of the VirB10–msfGFP foci and quantitative representation of the amount of foci present per cell, the images were also background-subtracted by a rolling-ball correction using ImageJ. µZ-plane stacks were obtained from a 0.5-µm HC PL APO 100×/1.4 oil ph3 objective (Leica) and a GFP excitation–emission band-pass filter cube (Ex.: 470/40, 510–550 nm; Em.: 525/50; Leica). To increase spatial resolution, 100-0.5-µm Z-planes were stacked from a 0.5-µm step and 10% of the frames were obtained from the median plane of focus. This allowed for a better signal-to-noise ratio of the VirB10–msfGFP foci and quantitative representation of the amount of foci present per cell, the images were also background-subtracted by a rolling-ball correction using ImageJ. µZ-plane stacks were obtained from a 0.5-µm HC PL APO 100×/1.4 oil ph3 objective (Leica) and a GFP excitation–emission band-pass filter cube (Ex.: 470/40, 510–550 nm; Em.: 525/50; Leica). To increase spatial resolution, 100-0.5-µm Z-planes were stacked from a 0.5-µm step and 10% of the frames were obtained from the median plane of focus. This allowed for a better signal-to-noise ratio of the VirB10–msfGFP foci and quantitative representation of the amount of foci present per cell, the images were also background-subtracted by a rolling-ball correction using ImageJ. µZ-plane stacks were obtained from a 0.5-µm HC PL APO 100×/1.4 oil ph3 objective (Leica) and a GFP excitation–emission band-pass filter cube (Ex.: 470/40, 510–550 nm; Em.: 525/50; Leica). To increase spatial resolution, 100-0.5-µm Z-planes were stacked from a 0.5-µm step and 10% of the frames were obtained from the median plane of focus. This allowed for a better signal-to-noise ratio of the VirB10–msfGFP foci and quantitative representation of the amount of foci present per cell, the images were also background-subtracted by a rolling-ball correction using ImageJ. µZ-plane stacks were obtained from a 0.5-µm HC PL APO 100×/1.4 oil ph3 objective (Leica) and a GFP excitation–emission band-pass filter cube (Ex.: 470/40, 510–550 nm; Em.: 525/50; Leica). To increase spatial resolution, 100-0.5-µm Z-planes were stacked from a 0.5-µm step and 10% of the frames were obtained from the median plane of focus. This allowed for a better signal-to-noise ratio of the VirB10–msfGFP foci and quantitative representation of the amount of foci present per cell, the images were also background-subtracted by a rolling-ball correction using ImageJ. µZ-plane stacks were obtained from a 0.5-µm HC PL APO 100×/1.4 oil ph3 objective (Leica) and a GFP excitation–emission band-pass filter cube (Ex.: 470/40, 510–550 nm; Em.: 525/50; Leica). To increase spatial resolution, 100-0.5-µm Z-planes were stacked from a 0.5-µm step and 10% of the frames were obtained from the median plane of focus. This allowed for a better signal-to-noise ratio of the VirB10–msfGFP foci and quantitative representation of the amount of foci present per cell, the images were also background-subtracted by a rolling-ball correction using ImageJ. 1706518; 1:5,000) was used for AbVirB7 and AbVirB8 with BCIP (VWR 0885) and NRT (Sigma-Aldrich N6876) for detection, and secondary goat anti-rabbit IgG-AP conjugate (Bio Rad) was used for all AbVirB8, AbVirB10 and AbVirB10 before visualization using an Odyssey imaging system (Li-Cor).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Density map is available at EMDB with accession code EMD-0089. Atomic model is available in Protein Data Bank with accession code 4GB. NMR data are available at BMRB with accession number 27342. All other data supporting the findings of this study are available from the corresponding authors upon request.

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Author contributions

G.G.S. cloned, expressed and purified the *X. citri* T4S system core complex. G.G.S., A.C. and R.V.P prepared and collected negative-staining EM data. G.G.S., T.R.D.C. and R.V.P determined the sample preparation conditions for cryo-EM. T.R.D.C. prepared cryo-EM grids used for data collection (with G.G.S.), collected cryo-EM data, performed the image analysis and carried out the EM reconstructions. G.G.S. built and refined the model. G.G.S., W.C. and D.P.S. carried out the mutagenesis work. W.C. performed and analysed the biological assays and the microscopy analysis. G.G.S. carried out the immunoblotting analysis. L.C.O., D.P.S. and R.V.P. performed and collected negative-staining EM data. G.G.S. cloned, expressed and purified the Solu-EM for data collection (with G.G.S.), collected cryo-EM data, performed the image analysis and carried out the EM reconstructions. G.G.S. built and refined the model. G.G.S., W.C. and D.P.S. carried out the mutagenesis work. W.C. performed and analysed the biological assays and the microscopy analysis. G.G.S. prepared the figures. G.G.S., T.R.D.C., A.C. and G.W. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Experimental design

1. Sample size
Describe how sample size was determined.

Samples sizes were not predetermined for CryoEM due to the nature of the very large CryoEM dataset consisting of 1469 micrographs in movie mode (collected at eBIC facility at the Diamond Synchrotron) containing hundreds of thousands of particles as described in the Methods. Sample sizes were not predetermined for the bacterial killing assays given the large number of mutants tested. In most cases two or more isolates of each mutant was tested, giving essentially equivalent results. For quantification of GFP tagged T4S systems and for measurement of total cellular fluorescence, samples sizes were not predetermined due to the very large number of cells analyzed per mutant strain: on average 2,145 cells (minimum = 496 cells, maximum = 7,329 cells).

2. Data exclusions
Describe any data exclusions.

CryoEM analysis naturally involves the exclusion of data during processing based on image quality using criteria automatically determined by the software (for example, Gctf, Relion and CryoSparc). These procedures are described in the Methods.

3. Replication
Describe the measures taken to verify the reproducibility of the experimental findings.

The statistical analyses for the CryoEM and NMR data collections and the CryoEM model are provided in the relevant tables (Supplementary Table 1 and Supplementary Table 3) and in the Methods.

For the bacterial killing experiments, assays were performed 3 or 4 times for each mutant, 9 times for the X.citri VirB10-msfGFP control and 27 times for the wild-type X. citri control and the average of the resulting killing curves are presented with their standard deviations in Supplementary Table 4. To control for putative secondary modifications in the mutant’s genetic background beyond the targeted loci, wild-type revertant strains obtained during the selection of each mutant were all confirmed to have wild-type killing efficiencies. The A572 values were processed using RStudio software and plotted using the ggplot2 package. Background intensities obtained from the mean of A572 values of two repeats containing only E. coli cells were subtracted from the data series. Hereafter, each series was normalized to the initial OD600 values from each X. citri culture to correct for small differences in OD600 after overnight growth (Note that no significant difference in growth rate could be detected for the mutants, except for mutant VirB10ΔQ175-D182 which also showed smaller cell sizes). Next, the initial A572 value at time-point 0 minutes was subtracted from all subsequent time-points to correct for small differences in initial A572 measurements. Finally, each set of mutants was normalized to their wild-type counterparts, leading to the final figures shown with A572=0 serving as the E. coli control baseline. Additionally, to produce a single numerical comparison of the data, the mean slope (obtained by calculating the discrete first derivative) between time-point 50 and 100 minutes of the obtained normalized A572 curves of mutant and wild-type cells were compared to obtain the fraction of killing efficiency of each mutant compared to the wild-type X. citri virB10-msfGFP control.

To obtain a quantitative representation of the amount of foci present per cell, the images were analyzed using the MicrobeJ software package, providing an automated detection method of the amount of foci and their location in the different strains. X. citri virB10-msfGFP wild-type images were used to optimize MicrobeJ parameters for automated detection of the foci. Identical parameters were used for all images and visual inspection was used to confirm detection accuracy. All mutants were imaged and analyzed together and on two
separate occasions after which data sets were combined. On average, 2,145 cells were analyzed per mutant strain (minimum = 496 cells, maximum = 7,329 cells). For quantifiable visualization purposes, a multi-colored lookup table was applied to more easily represent differences in intensity levels and a representative image of X. citri virB10-msfGFP is provided in Fig. 5a, obtained after a blind deconvolution of the obtained Z-stacks (Leica LAS X software).

The above obtained images were also used to measure the average total fluorescence intensities of all the mutants using identical software packages as described above for X. citri virB10-msfGFP foci detection. This allowed for an independent measure of the VirB10-msfGFP levels and can be compared to the results observed in western blots presented in Fig. 6 and Supplementary Fig. 7b.

All attempts at replication in the bacterial killing assays and microscopy analyses were successful, within experimental error.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

This is not relevant to the study.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

No blinding was performed in the structural analysis nor in the phenotype analysis of the set of X. citri mutants since, in both cases, reported parameter values were calculated automatically or semi-automatically via the employed software as described in the Methods (see also item 3, Replication, above).

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a | Confirmed
---|---
☐ | ✗ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐ | ✗ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐ | ✗ A statement indicating how many times each experiment was replicated
☐ | ✗ The statistical test(s) used and whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
☐ | ✗ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐ | ✗ Test values indicating whether an effect is present
Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.
☐ | ✗ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☒ | ✗ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

7. Software

Describe the software used to analyze the data in this study.

Gctf (Zhang, 2016)
https://www.mrc-lmb.cam.ac.uk/kzhang/

MotionCor2 (Zheng et al., 2017)
http://msg.ucsf.edu/em/software/motioncor2.html

RELION v2.0 (Scheres, 2012)
http://www2.mrc-lmb.cam.ac.uk/relion/index.php/Main_Page

CryoSPARC v0.2.36 (Punjani et al., 2017)
https://cryosparc.com

NMRPipe (Delaglio et al., 1995)
https://www.ibbr.umd.edu/nmrpipe/index.html
Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

All unique materials are readily available from the authors or from third party commercial distributors.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

To visualize the effect in the expression, accumulation and stability of the Xac core complex proteins (VirB7, VirB9 and VirB10), western blot assays were performed using total protein extract for the wild-type and each mutant strain analyzed. Polyclonal antibodies specific for each protein were used. See reference <doi:10.1371/journal.ppat.1002031> for the protocol used to produce antibodies against VirB7, VirB8, VirB9 and VirB10. The rabbit anti-GFP antibody was purchased from Sigma-Aldrich (Cat #G1544, lot #077M4827V). The following dilutions for primary antibodies were used: VirB7 (AbVirB7; 1:20,000 dilution), VirB8 (AbVirB8; 1:10,000), VirB9 (AbVirB9; 1:4,000), VirB10 (AbVirB10; 1:5,000) and GFP (AbGFP; 1:6,000) were used. Secondary goat anti-rabbit IgG-AP conjugate (Bio Rad, cat 1706518, lot 94532; 1:5,000 dilution) was used for AbVirB7 and AbVirB8 with BCIP (VWR, 0885) and NBT (Sigma-Aldrich, cat N6876) for detection, and secondary goat anti-rabbit IgG-IRDye® 800CW (Li-Cor, cat 32211, lot C40325-02; 1:8,000 dilution) was used for AbVirB9, AbVirB10 and AbGFP prior to visualization using an Odyssey imaging system (Li-Cor).

Validation of the antibodies was performed using the total cell lysates of i) X. citri cells in which the relevant genes were knocked out (negative control), ii) wild-type X. citri cells (positive control), iii) E. coli cells (negative control) and iv) E. coli cells expressing the relevant recombinant protein (positive control).
10. Eukaryotic cell lines
   a. State the source of each eukaryotic cell line used. No eukaryotic cell lines were used.
   b. Describe the method of cell line authentication used. No eukaryotic cell lines were used.
   c. Report whether the cell lines were tested for mycoplasma contamination. No eukaryotic cell lines were used.
   d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use. No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
   Provide all relevant details on animals and/or animal-derived materials used in the study. No animals were used.

Policy information about studies involving human research participants

12. Description of human research participants
   Describe the covariate-relevant population characteristics of the human research participants. No human subjects were used.