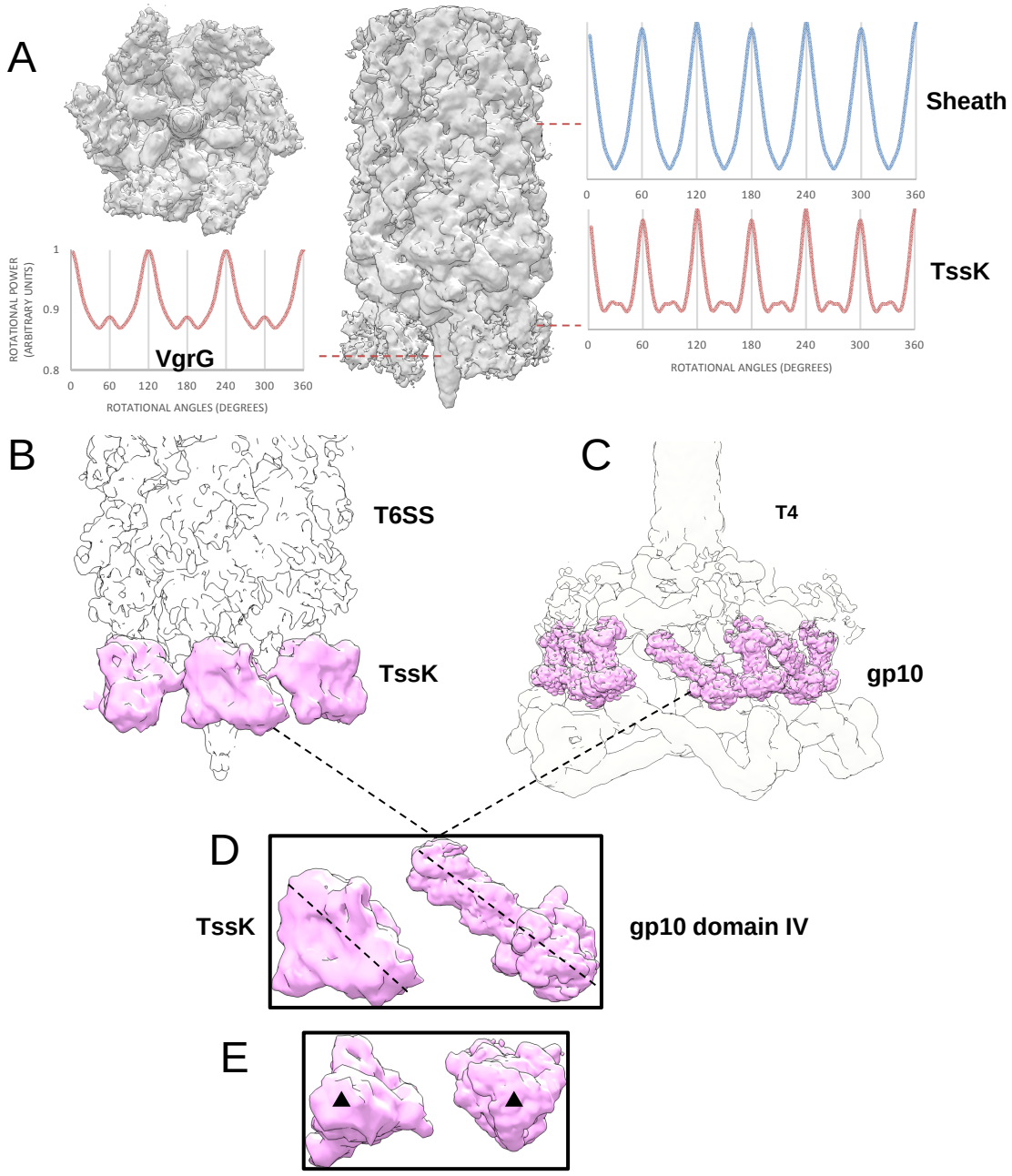
Table of contents

Appendix Fig. S1



Symmetry analysis, localized connector refinement and homology to T4 gp10.

(A). Bottom and side views of the 3-fold averaged sheath-baseplate reconstruction. Rotational power spectra of the cross sections through the sheath, wedge, and spike regions.

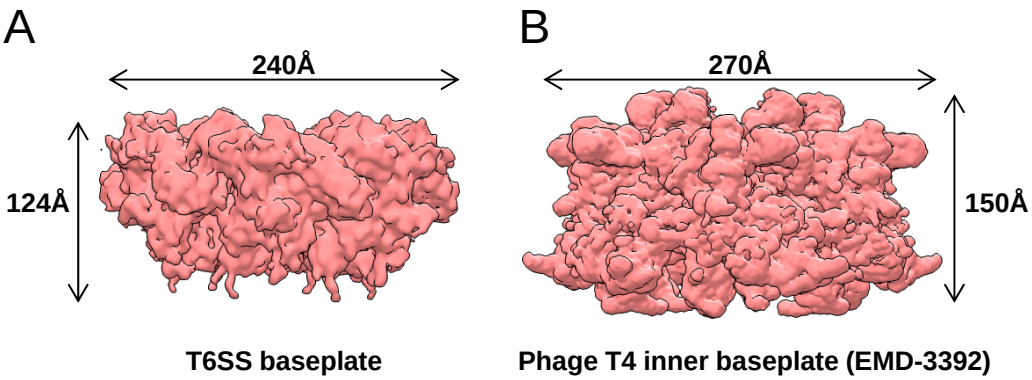
(B). Tilted view of the composite baseplate cryo-EM reconstruction, except for the disordered connector density, replaced by six copies of locally refined reconstruction colored in pink.

(C). Tilted view of the 12 Å T4 baseplate cryo-EM reconstruction (EMD-1048), with fitted upper peripheral baseplate (EMD-3394) colored in pink.

(D). Side views of the TssK connector protein and domain IV of T4 gp10 with three-fold symmetry axis highlighted with black dashed lines.

(E). View along three-fold symmetry axis of (D).

Appendix Fig. S2

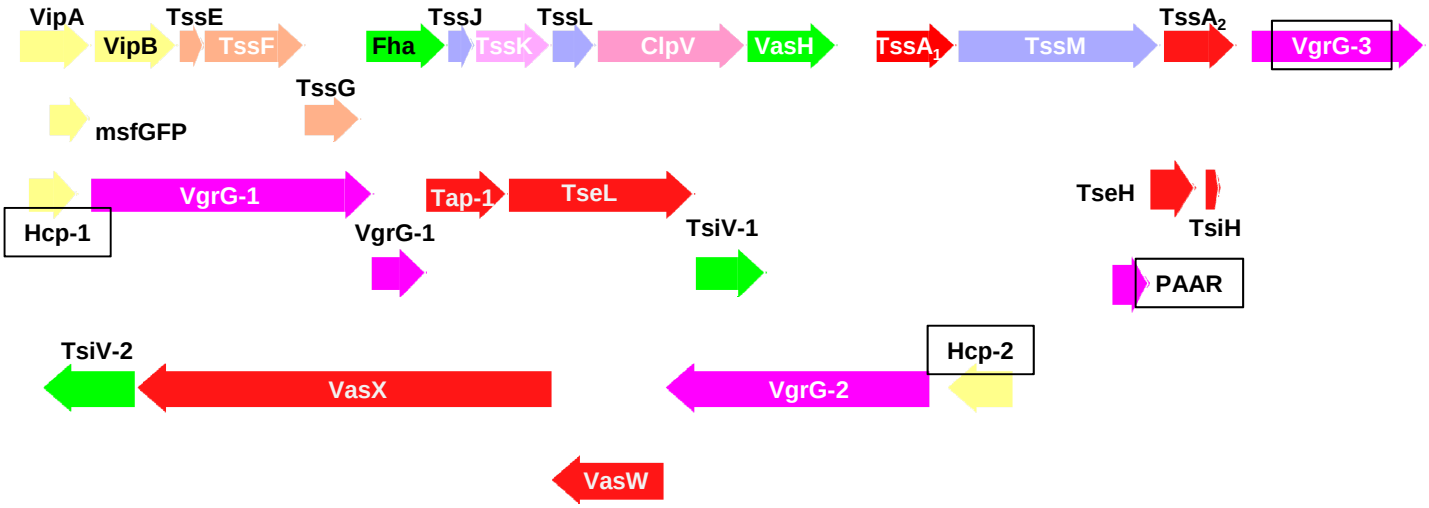


Comparison of the T6SS baseplate with the T4 phage inner baseplate.

(A). Extracted putative baseplate of 8 Å T6SS sheath-baseplate reconstruction.

(B). Cryo-EM reconstruction of the T4 inner baseplate (EMD-3392) low-pass filtered to 8 Å**.**

Appendix Fig. S3



Schematic representation of the genomic region encoding T6SS from *Vibrio cholerae*.

Main cluster with VgrG-3, and three auxiliary clusters – Hcp-1, Hcp-2 and PAAR are shown. Baseplate, membrane complex, cap, effectors, adaptors, immunity and regulatory proteins are shown.

Appendix Table S1. Cryo-EM data collection and processing.

<table>
<tr><th rowspan="3">T6S Part</th><th rowspan="3">Symmetry</th><th colspan="2">Movies</th><th colspan="5">Particles</th><th rowspan="3">Pixel size, Å</th><th rowspan="3">Resolution, Å</th><th rowspan="3">Software</th></tr>
<tr><th rowspan="2">Non Hcp lim.</th><th rowspan="2">Hcp lim.</th><th colspan="3">Non Hcp lim.</th><th colspan="2">Hcp lim.</th><th rowspan="2">Final 3DR Combined</th></tr>
<tr><th>Picked</th><th>After 2D</th><th>3D</th><th>Picked</th><th>After 2D</th></tr>
<tr><td>Baseplate</td><td>C6/C3</td><td rowspan="2">6,603</td><td rowspan="2">2,599</td><td rowspan="2">21,446</td><td rowspan="2">8,309</td><td>2,660</td><td rowspan="2">12,832</td><td rowspan="2">3,241</td><td>1,265</td><td rowspan="3">2.12</td><td>8/11</td><td rowspan="2">MotionCorr2, Gctf, Scipion, RELION1.4, 2</td></tr>
<tr><td>Distal end</td><td>C6</td><td>3,710</td><td>3,710*</td><td>7.5</td></tr>
<tr><td>TssK</td><td>C6-C1 relaxation</td><td colspan="6">-</td><td>3,600</td><td>10</td><td>RELION2</td></tr>
</table>

* Final 3D refinement with particles from non-Hcp limited cells only.

Appendix Table S2. Fitting of X-ray crystallographic structures into cryo-EM density maps of baseplate and distal end.

Model	PDB	% atoms	Correlation coefficient
Sheath ring 1	5MXN	87	0.87
Ring 1 refitted		90	0.89
Ring 2		86	0.86
Ring 3		89	0.88
Ring 4		91	0.89
Ring 5		91	0.90
VgrG/PAAR C6	4MTK/4JIV	88	0.88
VgrG/PAAR C3		94	0.93
Ring N (topmost)	5MXN	76	0.79
Ring N refitted		84	0.86
Ring (N-1)		90	0.87
Hcp N (topmost)	5MXN*	60	0.87
Hcp N-1		77	0.90
Hcp N-2		90	0.95
VipA/VipB only into distal end	5MXN**	73	0.82
TssK	5M30	66	0.92

* Hcp tube and **VipA/VipB sheath models were extracted from complete sheath-tube model (PDB 5MXN).

Appendix Table S3. Strains used in this study, related to Material and Methods.

Organism	Genotype	Plasmid	Relevant features	Source
V. cholerae 2740-80	lacZ$^-$, Str^r, *vipA-msfGFP*		C-terminal chromosomal fusion of *msfGFP* to *vipA*	(Kudryashev et al., 2015)
	lacZ$^-$, Str^r, *ΔvipA, ΔflgG*	pBAD24-*vipA*	Complementation of *vipA* deletion from inducible vector; Amp^r	(Brackmann et al., 2017)
	lacZ$^-$, Str^r, *ΔvipA, ΔflgG*	pBAD24-*vipA-N3*	Complementation of *vipA* deletion from inducible vector; Amp^r	
	lacZ$^-$, Str^r, *vipA-msfGFP, Δhcp1, Δhcp2*		Deletion of both *hcp* variants in *vipA-msfGFP* background	(Vettiger et al., 2016)
	lacZ$^-$, Str^r, *vipA-msfGFP, Δhcp1, Δhcp2, ΔflgG*		*flgG* deletion in *hcp* mutant background	this study
	lacZ$^-$, Str^r, *vipA-msfGFP, Δhcp1, Δhcp2, ΔflgG*	pBAD24-*hcp2*	Complementation of *hcp* deletion from inducible vector; Amp^r	
	lacZ$^-$, Str^r, *vipA-N3-msfGFP, Δhcp1, Δhcp2, ΔflgG*		Chromosomal integration of *vipA-N3-msfGFP* in *hcp / flgG* deletion background	
	lacZ$^-$, Str^r, *vipA-N3-msfGFP, Δhcp1, Δhcp2, ΔflgG*	pBAD24-*hcp2*	Complementation of *hcp* deletion in *vipA-N3-msfGFP* background from inducible vector; Amp^r	
E. coli SM10 λ pir	Km^r, thi-1, thr, leu, tonA, lacY, supE, recA::RP4-2-Tc::Mu, pir	pWM91	Allelic replacement vector used for all in-frame deletions by conjugation; *sacB*, Amp^r/Gent^r	
DH5α λ pir	F$^-$, endA1, glnV44, thi-1, recA1, relA1, gyrA96 deoR, nupG, Φ80d*lacZ*ΔM15, (*lacZYA-argF*)U169, hsdR17(rK$^-$ mK$^+$), λ$^-$		Cloning strain	