Supplementary information Multiple conformations facilitate PilT function in the type IV pilus Matthew McCallum *et al.*

Protein (PDB)	PDB Reference	System	Inter-chain C	a distance (Å)*	Corresponding	Pattern of
			Т221–Н230	T221–L269	Interface	Interfaces
Methylated PilT ^{Gm}	60JY ^{Herein}	T4aP	5.8 12.2	14.1 8.9	0	OCOCOC
Low ADP PilT ^{Gm}	60JZ ^{Herein}	T4aP	5.8 13.6	14.0 8.3	C O	OCOCOC
High ADP PilT ^{Gm}	60K2 ^{Herein}	T4aP	5.6 13.6	14.2 8.2	C Q	OCOCOC
ATP bound PilT ^{Gm}	60JX ^{Herein}	T4aP	6.1	12.3	C	CCCCCC
ANP/ADP bound PilT ^{Gm}	60KV ^{Herein}	T4aP	5.4 11.6	13.7	C O	CCOCCO
PilT ^{Pa}	3.IVV ¹	T4aP	5.7	14.5	C	CCCCCC
PilT ^{Pa}	3JVU ¹	T4aP	5.5	13.8	C	CCCCCC
PilT ^{Aa}	2GSZ ²	T4aP	7.6	19.4	C	00C00C
DilTGs	57E03	T4oP	15.8	9.9	0	000000
PILI Co	$\frac{3ZFQ^3}{2EWV^2}$	T4aP	12.0	12.7	<u> </u>	<u>vvvvv</u>
DilTAa	2E W V 2EWW2	T4ar T4aP	10.0	13.7	<u>л</u> V	
1 11 1	21.00 00	1441	5.0	20.6	<u>л</u> С	ΛΛΛΛΛΛ
PilB ^{Gm}	5TSH ⁴	T4aP	22.5	20.8	0	CCOCCO
PilBGm	5TSG ⁴	T4aP	6.6	20.2	С	CCOCCO
TID	5150	1741	21.3	9.3	0	000000
DilDTt	51755	T/oP	6.1	20.0	С	CCOCCO
TID	5115	1441	20.6	9.8	0	ccocco
PilB ^{Tt}	50IU ⁶	T4aP	6.1 21.4	20.2	C	CCOCCO
			6.1	20.5	<u> </u>	
PilB ^{Gs}	5ZFR ³	T4aP	21.4	9	õ	CCOCCO
GspE	4KSR ⁷	T2S	7.4	19.6	С	CCOCCO
C.F.	42.007	T25	21.4	9.4	0	000000
GspE	4K887	128	5.0	21.4	<u> </u>	
FlaI	4II7 ⁸	Archaellum	8.6 14.7	19.5 7.9	0	OCOCOC
FlaI	4IHQ ⁸	Archaellum	7.0	18.5	С	CCOCCO
		Arabaaal	7.8	16.1	<u> </u>	
Archaeal GspE	$2OAP^9$	T2S	11.4	8.8		OCOCOC
ļ		Archaeal	7.6	16.1	C C	
Archaeal GspE	20AQ ⁹	T2S	11.9	9.8	0	OCOCOC
VirB11	1NI V ¹⁰	T4aS	5.0	13.0	C C	CCCCCC
VirB11	2PT7 ¹¹	T4aS	6.0	14.3	C	CCCCCC
VirB11	1NLZ ¹⁰	T4aS	5.0	13.0	C	CCCCCC
VirB11	10PX ¹⁰	T4aS	5.0	13.0	С	CCCCCC
	(CED ¹²		6.9	15.0	C	
DotB	00ED	T4bS	14.4	8.5	0 0	OCOCOC
DotB	6GEF ¹²	T4bS	7 16.8	13.1 9.2	C O	CCOCCO
CrvoEM structures	presented here	in				
D.1177C-	COLL	m (m	5.4	13.6	C	000000
PilT ^{om}	6OLL ^{Herein}	T4aP	11.4	7.7	Ō	OOCOOC
PilT ^{Gm}	60LK ^{Herein}	T4aP	6.0	13.4	С	OCOCOC
	(OLK		12.6	7.9	0	000000
Pillom	60LM ^{Herein}	T4aP	6.2	12.4	C	CCCCCC
PilB ^{Gm}	60LJ ^{Herein}	T4aP	6.4	20.2	C	CCOCCO
			22.7	9.4	0	

Supplementary Table 1. Defining the open- and closed-interfaces across PilT/VirB11-like family member structures.

*Mean distance reported in the case when multiple open- or closed-interfaces are present in the same hexamer. In homologs of PilT^{Gm}, residues that align with T221, H230, and L269 from PilT^{Gm} are used: respectively, T233, H242, and L281 for PDB 2EWV, 2EWW, and 2GSZ; T220, H229, and L268 for PDB 3JVV and 3JVU; T221, H230, and L269 for PDB 5ZFQ; T411, H420, and R455 for PDB 5TSH, 5TSG, 5ZFR, and 6OLJ; T735, H744, and R779 for PDB 51T5; T245, H254, and R289 for PDB 50IU; T251, H260, and L299 for PDB 6GEB; T266, H275, and L314 for PDB 6GEF; T350, H359, and R394 for PDB 4KSR and 4KSS; S263, H273, and H318 for PDB 1NLY, 2PT7, 1NLZ, and 1OPX; T356, H365, and M399 for PDB 2OAP and 2OAQ; and T351, H360, and L395 for PDB 4II7 and 4IHQ. **The open-interface defined as T221–H230 distance > 12 Å and T221–L269 distance < 11 Å. The closed-interface defined as T221–

H230 distance < 9 Å and T221–L269 distance > 11 Å.

Primers	Sequence	Reference
P168	TATATATACATATGGCCAACATGCATCAGC	This Study
P169	TATATACTCGAGTTATCTCATGGGGGGGGGG	This Study
P220	TATAATGGTACCTACCGAGCTGCTCGCCTT	This Study
P221	TATATATCTAGAATCAGAAGTTTTCCGGGATCTT	This Study
E204A fwd	CATCCTGGTCGGCGCGATG	This Study
E204A rev	CATCGCGCCGACCAGGATG	This Study
H229A fwd	TCGGCACCCTGGCCACCACCTCG	This Study
H229A rev	CGAGGTGGTGGCCAGGGTGCCGA	This Study
R239A fwd	ACACGTCGACCACCGCGTCGATGGTCTTC	This Study
R239A rev	GAAGACCATCGACGCGGTGGTCGACGTGT	This Study
E219K fwd	GTGGCCGGTCTTCGCCGCGGTCA	This Study
E219K rev	TGACCGCGGCGAAGACCGGCCAC	This Study
D31K fwd	ATGATCCGGGTGAATGGCGATGTACG	This Study
D31K rev	CGTACATCGCCATTCACCCGGATCAT	This Study
F258A fwd	CTCGATGCTCTCCGCGTCGCTGCAAT	This Study
E250A rev		This Study
R294F fwd	GCGCGACCTTGTCCTCCTCGATCAGGTTGCGGAT	This Study
R204E rev		This Study
D242K fived	GGCCGGGAACACCTTGACCACCCGGTC	This Study
D242K Iwd	GACCGGGTGGTCAAGGTGTTCCCGGCC	This Study
T216P find		This Study
T216R_row		This Study
P123D fred		This Study
R123D_IWU	TTCACACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	This Study
K125D rev		This Study
K58A_IWU		This Study
K58A lev		This Study
K58Q IWU		This Study
K50Q fev		This Study
K58R IWd		This Study
K58K rev	GAAGICCIIGCGCIGACGGICGIICAIGAIGIC	This Study
Strains	Description	Keierence
E. coli IOPI0	F', mcrA Δ (mrr-hsdRMS-mcrBC) ϕ 80lacZ Δ M15 Δ lacX/4 nupG recA1 araD139 Δ (ara-leu)7697 galE15 galK16 rpsL(Str ^R) endA1 λ^{-}	Invitrogen
E. coli BL21-CodonPlus® cells	Expression strain; $F^- ompT hsdS$ (r $B^- mB^-$) dcm ⁺ Tet ^r gal λ (DE3) endA [argU proL Cam ^r]	Stratagene
E. coli BTH101	BACTH coexpression strain; F ⁻ , <i>cya-99, araD139, galE15, galK16, rpsL1, hsdR2 mcr41 mcrB1</i>	Euromex
P. aeruginosa PAO1 nilT::FRT	Retraction deficient mutant with FRT disruption in <i>nilT</i> gene	13
Plasmids	Description	Reference
pET28a::PilTPA	PilT from <i>P</i> aeruginosa for expression in <i>E</i> coli	14
pET28a::PilB ^{Gm}	PilB from G. metallireducens for expression in E. coli	4
pET28a::PilT ^{Gm}	PilT4 from <i>G</i> metallireducens for expression in <i>E</i> coli	This Study
pBADGr	pBAD plasmid with gentamicin resistance	13
pBADGr::PilT ^{PA}	PilT from <i>P</i> aeruginosa for expression in <i>P</i> aeruginosa	This Study
E204A, H229A, R239A, E219K,	pBADGr::PilT ^{PA} constructs with individual site mutations	This Study
D31K, E258A, R294E, D242K,	1	2
T216R, R123D, K58A, K58Q,		
K58R		
pUT18C:PilC ^{PA}	PilC from P. aeruginosa BACTH construct	15
pUT18C:PilT ^{PA}	PilT from P. aeruginosa BACTH construct	15
pKT25:PilT ^{PA}	PilT from P. aeruginosa BACTH construct	15
E204A, H229A, R239A, E219K,	pKT25:PilT ^{PA} constructs with individual site mutations	This Study
D31K, E258A, R294E, D242K,		
1216R, R123D, K58A, K58Q, K58R		
Antibodies	Description	Reference
a-PilT	Purified rabbit IgG antibody made with PilT from P. aeruginosa	16

Sur	oplementary	v Table 2: 1	Primers.	strains.	plasmids.	and	antibodies	used in	this	study
~ ~	promonout.	,		501 6611159	presidente	,	unuouuo	abea m	UTTTO I	Sectory.

Relevant ligand SO.2· Partial occupancy ADP Full occupancy ADP ATP ANP and ADP Data collection	Crystal structure name	Methylated OCOCOC PilT	Partial occupancy ADP OCOCOC PilT	Full occupancy ADP OCOCOC PilT	CCCCCC PilT	CCOCCO PilT		
Data collection Facility CLS NSLS-II NSLS-II APS CLS Beamline 08-ID-1 17-ID-2 17-ID-1 23-ID-B 08-ID-1 Wavelength (Å) 0.97949 1.282140 0.999614 1.033202 0.97949 Space group P22.22. P2.2.21 P2.2.22. P6 P22.22. a, b, c (Å) 115.8, 119.01, 178.3 112.3, 121.1, 187.1 111.8 121.0 185.9 190.2, 190.2, 60.5 98.5, 127.1, 187.3 a, b, c (Å) 48-3.3 29-3.0 29-3.3 48-1.9 48-4.0 (34-3.3) (3.1-3.0) (3.4-3.3) (2.0-1.9) (4.1-4.0) Total Reflections 281393 620489 258149 1037608 127072 Unique Reflections 37753 (3658 4974 (4342) 38771 (3597) 99891(942) 38507 (174.5) Completeness (%) 100 (99) 98 (85) 99 (94) 100 (100) 98 (10)* Mean (/ a I 12.6 (2.3) 12 (2.2) 7.5 (1.3) 10.6 (1.3) 4.0 (1.6) Rest (%)*	Relevant ligand	SO4 ²⁻	Partial occupancy ADP	Full occupancy ADP	ATP	ANP and ADP		
Facility CLS NSLS-II NSLS-II APS CLS Beamline $08-1D-1$ $17-1D-2$ $17-1D-1$ $23-1D-8$ $08-1D-1$ Space group $P2_12_12$ $P2_12_12$ $P2_12_2$ $P6$ $P2_22_2$ $P2_2^2$ $P2_2^2$ $P2_2^2$ $P2_2^2$ $P2_2^2$ $P2_2^2$ $P2_2^2$ $P2_2^2$ $P2_2^2$ $P2_2^2^2$ $P2_2^2^2^2$ $P2_2^2^2^2$ $P2_2^2^2^2^2$ $P2_2^2^2^2^2^2$ $P2_2^2^2^2^2^2^2^2^2^2^2^2^2^2^2^2^2^2^2$	Data collection							
Beamline 08-ID-1 17-ID-2 17-ID-1 23-ID-8 08-ID-1 Wavelength (Å) 0.97949 1.282140 0.999614 1.033202 0.97949 Space group $P^2_{1,2;1}$ $P^2_{2,1;2;1}$ $P^2_{2,1;2;1}$ $P^2_{0,2;2;1}$ $P^2_{0,2;2;1;1}$ $P^2_{0,2;2;1}$	Facility	CLS	NSLS-II	NSLS-II	APS	CLS		
Wavelength (Å) 0.97949 1.282140 0.999614 1.033202 0.97949 Space group $P2_{12}_{12}_{11}$ $P2_{12}_{11}_{11}$ $P2_{12}_{12}_{11}$ $P2_{12}_{11}_{11}$ $P2_{11}_{11}$ $P2_{1$	Beamline	08-ID-1	17-ID-2	17-ID-1	23-ID-B	08-ID-1		
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Wavelength (Å)	0.97949	1.282140	0.999614	1.033202	0.97949		
a, b, c (Å) 115.8, 119.01, 178.3 112.3, 121.1, 187.1 111.8, 121.0, 185.9 190.2, 190.2, 40.5 98.5, 127.1, 187.3 a, β, γ (°) 90, 90, 90 94 48.4.0 14.4.0 12.11, 18.7.1 111.8, 121.0 185.9 1037608 12072 12072 Unique Reflections 281393 620489 258149 1037608 12072 <td>Space group</td> <td>$P2_{1}2_{1}2_{1}$</td> <td>$P2_{1}2_{1}2_{1}$</td> <td>$P2_{1}2_{1}2_{1}$</td> <td><i>P</i>6</td> <td>$P2_{1}2_{1}2_{1}$</td>	Space group	$P2_{1}2_{1}2_{1}$	$P2_{1}2_{1}2_{1}$	$P2_{1}2_{1}2_{1}$	<i>P</i> 6	$P2_{1}2_{1}2_{1}$		
a , β , γ (°) 90, 90, 90 90, 90, 90 90, 90, 90 90, 90, 90 90, 90, 90 Resolution (Å) 48-3.3 29-3.0 29-3.3 48-1.9 48-4.0 (3.4-3.3) (3.1-3.0) (3.4-3.3) (2.0-1.9) (4.1-4.0) Total Reflections 281393 620489 258149 1037608 127072 Unique Reflections 37735 (3658) 49747 (4342) 38771 (3597) 99891(9942) 38507 (1745) Redundancy 7.4 (7.6) 12.5 (12.1) 6.7 (6.0) 10.4 (10.3) 3.3 (0.6) Completeness (%) 100 (99) 98 (85) 99 (94) 100 (100) 98 (10)* Mean / a_1 1 12.6 (2.3) 12 (2.2) 7.5 (1.3) 10.6 (1.3) 4.0 (1.6) R_{sm} (%)** 16 (105) 16 (84) 16 (110) 12 (143) 28 (112) CC* (%)* 100 (93 100 (95) 100 (87) 100 (89) 100 (86) Remach Raice (%)* 21.0 / 24.5 19.3 / 23.1 21.2 / 25.43 18.4 / 21.2 24.8 / 27.6	a, b, c (Å)	115.8, 119.01, 178.3	112.3, 121.1, 187.1	111.8 121.0 185.9	190.2, 190.2, 60.5	98.5, 127.1, 187.3		
Resolution (Å) 48-3.3 29-3.0 29-3.3 48-1.9 48-4.0 (3.4-3.3) (3.1-3.0) (3.4-3.3) (2.0-1.9) (4.1-4.0) Total Reflections 281393 620489 258149 1037608 127072 Unique Reflections 37753 (3658) 49747 (4342) 38771 (3597) 99891(9942) 38507 (1745) Redundancy 7.4 (7.6) 12.5 (12.1) 6.7 (6.0) 10.4 (10.3) 3.3 (0.6) Completeness (%) 100 (99) 98 (85) 99 (94) 100 (100) 98 (10)* Mean I / o I 12.6 (2.3) 12 (2.2) 7.5 (1.3) 10.6 (1.3) 4.0 (1.6) $R_{sym} (\%)^*$ 160 (105) 16 (84) 16 (110) 12 (143) 28 (112) CC* (* $\phi)^*$ 100 (93) 100 (95) 100 (87) 100 (89) 100 (86) Reflnement 7 21.0 / 24.5 19.3 / 23.1 21.2 / 25.43 18.4 / 21.2 24.8 / 27.6 RMSD 0 0.005 0.009 0.008 0.011 0.009 Bond lengths (Å)<	α, β,γ(°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 120	90, 90, 90		
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Resolution (Å)	48-3.3	29-3.0	29-3.3	48-1.9	48-4.0		
Total Reflections 281393 620489 258149 1037608 127072 Unique Reflections 37753 (3658) 49747 (4342) 38771 (3597) 99891(9942) 38507 (1745) Redundancy 7.4 (7.6) 12.5 (12.1) 6.7 (6.0) 10.4 (10.3) 3.3 (0.6) Completeness (%) 100 (99) 98 (85) 99 (94) 100 (100) 98 (10)* Mean 1 / σ 1 12.6 (2.3) 12 (2.2) 7.5 (1.3) 10.6 (1.3) 4.0 (1.6) Rsme (%)** 100 (93) 100 (95) 100 (87) 100 (89) 100 (86) Refinement 21.2 / 25.43 18.4 / 21.2 24.8 / 27.6 RMSD 0.009 0.008 0.011 0.009 Bond lengths (Å) 0.005 0.009 0.008 0.011 0.009 Bond lengths (Å) 0.90 1.03 0.84 0.88 1.12 Ramachardraft 100 100 100 Favourd (%) 98 98 98 <td></td> <td>(3.4-3.3)</td> <td>(3.1-3.0)</td> <td>(3.4-3.3)</td> <td>(2.0-1.9)</td> <td>(4.1-4.0)</td>		(3.4-3.3)	(3.1-3.0)	(3.4-3.3)	(2.0-1.9)	(4.1-4.0)		
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Total Reflections	281393	620489	258149	1037608	127072		
Redundancy 7.4 (7.6) 12.5 (12.1) 6.7 (6.0) 10.4 (10.3) 3.3 (0.6) Completeness (%) 100 (99) 98 (85) 99 (94) 100 (100) 98 (10)* Mean I / σI 12.6 (2.3) 12 (2.2) 7.5 (1.3) 10.6 (1.3) 4.0 (1.6) Rsm (%)* 16 (105) 16 (84) 16 (110) 12 (143) 28 (112) CC* (%)** 100 (93) 100 (95) 100 (87) 100 (89) 100 (86) Remet / ////////////////////////////////////	Unique Reflections	37753 (3658)	49747 (4342)	38771 (3597)	99891(9942)	38507 (1745)		
Completeness (%) 100 (99) 98 (85) 99 (94) 100 (100) 98 (10)* Mean I / σ I 12.6 (2.3) 12 (2.2) 7.5 (1.3) 10.6 (1.3) 4.0 (1.6) R_{sym} (%)** 16 (105) 16 (84) 16 (110) 12 (143) 28 (112) CC* (%)** 100 (93) 100 (95) 100 (87) 100 (89) 100 (86) Refinement Newt / Rinec (%)* 21.0 / 24.5 19.3 / 23.1 21.2 / 25.43 18.4 / 21.2 24.8 / 27.6 RMSD Bond lengths (Å) 0.005 0.009 0.008 0.011 0.009 Bond lengths (Å) 0.90 1.03 0.84 0.88 1.12 Ramachandran ^a Favoured (%) 100 100 100 100 Coordinate error (Å) [§] 0.34 0.35 0.52 0.21 0.48 Atoms 16457 16569 16205 9562 16204 Protein 16236 16357 16031 8335 16010 Magnesium 0 0	Redundancy	7.4 (7.6)	12.5 (12.1)	6.7 (6.0)	10.4 (10.3)	3.3 (0.6)		
Mean $I / \sigma I$ 12.6 (2.3) 12 (2.2) 7.5 (1.3) 10.6 (1.3) 4.0 (1.6) $R_{Sym}(\%)^*$ 16 (105) 16 (84) 16 (110) 12 (143) 28 (112) CC* (%)** 100 (93) 100 (95) 100 (87) 100 (89) 100 (86) Refinement 24.8 / 27.6 RMSD 0.005 0.009 0.008 0.011 0.009 Bond lengths (Å) 0.005 0.009 0.008 0.011 0.009 Bond lengths (Å) 0.90 1.03 0.84 0.88 1.12 Ramachandran ⁴ 100 100	Completeness (%)	100 (99)	98 (85)	99 (94)	100 (100)	98 (10)*		
R_{sym} (%)** 16 (105) 16 (84) 16 (110) 12 (143) 28 (112) CC* (%)* 100 (93) 100 (95) 100 (87) 100 (89) 100 (86) Refinement	Mean $I / \sigma I$	12.6 (2.3)	12 (2.2)	7.5 (1.3)	10.6 (1.3)	4.0 (1.6)		
CC* $({\phi})^{**}$ 100 (93) 100 (95) 100 (87) 100 (89) 100 (86) Refinement	$R_{\rm Sym}$ (%)**	16 (105)	16 (84)	16 (110)	12 (143)	28 (112)		
Refinement Image: Constraint of the second se	CC* (%)**	100 (93)	100 (95)	100 (87)	100 (89)	100 (86)		
$R_{work} / R_{free} (\%)^{\dagger}$ $21.0 / 24.5$ $19.3 / 23.1$ $21.2 / 25.43$ $18.4 / 21.2$ $24.8 / 27.6$ RMSD Bond lengths (Å) 0.005 0.009 0.008 0.011 0.009 Bond lengths (Å) 0.90 1.03 0.84 0.88 1.12 Ramachandran [±]	Refinement			× - 4				
RMSD 0.005 0.009 0.008 0.011 0.009 Bond lengths (Å) 0.90 1.03 0.84 0.88 1.12 Ramachandran ⁴ 7 7 7 7 7 7 Favoured (%) 98 100	$R_{\text{work}} / R_{\text{free}} (\%)^{\dagger}$	21.0 / 24.5	19.3 / 23.1	21.2 / 25.43	18.4 / 21.2	24.8 / 27.6		
Coordinate error (Å)100100100100Atoms164571656916205956216204Protein162361635716031833516010Water1915012109912Magnesium00034ANP0000124ATP000930ADP0162162054Sulfate300000Ethylene Glycol00320Av. B-factors (Å ²)72.681.397.644.286.4Protein73.281.397.543.186.4Water26.165.770.854.784.6Ligands45.889.911935.787.1Deposited density and coordinate filesPDB ID60JY60JZ60K260KY	RMSD Bond lengths (Å) Bond angles (°) Ramachandran [‡] Favoured (%) Allowed (%)	0.005 0.90 98	0.009 1.03 98 100	0.008 0.84 98 100	0.011 0.88 98 100	0.009 1.12 98 100		
Coordinate error (A)s 0.34 0.53 0.52 0.21 0.48 Atoms 16457 16569 16205 9562 16204 Protein 16236 16357 16031 8335 16010 Water 191 50 12 1099 12 Magnesium 0 0 0 3 4 ANP 0 0 0 0 124 ATP 0 0 0 93 0 ADP 0 162 162 0 54 Sulfate 30 0 0 0 0 0 Attributer 162 162 0 54 0 Sulfate 30 0 0 0 0 0 Attributer 26.1 65.7 70.8 54.7 84.6 Ugands 45.8 89.9 119 35.7 87.1 Deposited density and coordinate files U U 60IX 60IX 60IX	Counting (1)	0.24	0.25	0.52	0.21	0.49		
Atoms 16427 16569 16203 9362 16204 Protein 16236 16357 16031 8335 16010 Water 191 50 12 1099 12 Magnesium 0 0 0 3 4 ANP 0 0 0 0 24 ATP 0 0 0 0 24 ADP 0 162 162 0 54 Sulfate 30 0 0 0 0 Ethylene Glycol 0 0 0 32 0 Av. B-factors (Å ²) 72.6 81.3 97.6 44.2 86.4 Protein 73.2 81.3 97.5 43.1 86.4 Water 26.1 65.7 70.8 54.7 84.6 Ligands 45.8 89.9 119 35.7 87.1 Deposited density and coordinate filesPDB ID $60JY$ $60JZ$ $60K2$ $60KY$	Atoma	0.54	0.35	0.52	0.21	0.48		
Protein 16236 16337 16031 6333 16010 Water 191 50 12 1099 12 Magnesium 0 0 0 3 4 ANP 0 0 0 0 124 ATP 0 0 0 93 0 ADP 0 162 162 0 54 Sulfate 30 0 0 0 0 Ethylene Glycol 0 0 32 0 Av. B-factors (Å ²) 72.6 81.3 97.6 44.2 86.4 Protein 73.2 81.3 97.5 43.1 86.4 Water 26.1 65.7 70.8 54.7 84.6 Ligands 45.8 89.9 119 35.7 87.1 Deposited density and coordinate files U 60/Y 60/Y 60/Y 60/Y	Atoms	16457	16257	16205	9362	16204		
Watch 191 30 12 1099 12 Magnesium 0 0 0 3 4 ANP 0 0 0 0 124 ATP 0 0 0 93 0 ADP 0 162 162 0 54 Sulfate 30 0 0 0 0 Ethylene Glycol 0 0 0 32 0 Av. B-factors (Å ²) 72.6 81.3 97.6 44.2 86.4 Protein 73.2 81.3 97.5 43.1 86.4 Water 26.1 65.7 70.8 54.7 84.6 Ligands 45.8 89.9 119 35.7 87.1 Deposited density and coordinate files U U GOX GOX GOX GOX	Water	10230	50	12	1000	12		
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Viact 20.1 00.7 70.0 34.7 84.0 Ligands 45.8 89.9 119 35.7 87.1 Deposited density and coordinate files PDB ID 60JY 60JZ 60K2 60JX 60KV	Water	26.1	65 7	70.8	547	84.6		
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Supplementally Table 5. Data concentri and remember statistics of the estat structure	Supplementary	/ Table 3. Da	ta collection ar	nd refinement	statistics of	of PilT c	rvstal	structure
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Note: Values in parentheses correspond to the highest resolution shell.

*, Atypical completeness of the CCOCCO PiIT structures reflects anisotropic truncation **, $R_{\text{Sym}} = \Sigma \Sigma |I - (I)| / \Sigma \Sigma I$, $R_{\text{Pim}} = \Sigma \sqrt{(1/(n-1))\Sigma} |I - (I)| / \Sigma \Sigma I$, and $\text{CC}^* = \sqrt{(2\text{CC}_{1/2}/(1+\text{CC}_{1/2}))}$ where $\text{CC}_{1/2}$ is the Pearson correlation coefficient of two half data sets as described elsewhere¹⁷

[†], $R_{\text{work}} = \Sigma ||F_{\text{obs}}| - k|F_{\text{calc}}|| / |F_{\text{obs}}|$ where F_{obs} and F_{calc} are the observed and calculated structure factors, respectively. R_{free} is the sum extended over a subset of reflections (5%) excluded from all stages of the refinement

§, Maximum-likelihood based Coordinate Error, as determined by PHENIX18

Supplementary	Table	4.	CryoEM	data	acquisition,	processing,	atomic	model	statistics	and
map/model dep	ositions.									

Data collection	1				Tilted data	a collection	All other samples				
Electron Micr	oscope				Titan	Krios	FEI Tecnai F20				
Tilt angle					4	0°	0°				
Camera					Falco	n 3EC	Gatan K2 Summit				
Voltage (kV)					3	00		200			
Nominal Mag	nification				75	000		25000			
Calibrated Pix	tel size (A)				1.	06			1.45		
Exposure rate	(e/pixel/s)				0	.8			5		
Exposure (e/A	<u>1</u> ²)				42	2.7			35		
Frames	•	_	_	_		<u></u>		4 11	30		
Image process	ing tion offered				Tilted data	A D C w2		All	other sampl	es	
CTE estimatic	n coftware	le		CTI	CIYOSP FEIND4 (rof	AKC V2	CTE)	Cr	TEEIND4		
Darticle select	ion software	2		CII	cryoSP	$APC x^2$	CIF)	07	UNTIND4)	
Classification	and refinen	- Jent softwa	re		cryoSP	ARC v2		CT	VOSPARC V2	,	
Classification	and remien	ient sonwa			cryosi	ARC V2		CI j	9051 AKC 72		
		DUTC				DIUDCm	DUTC	D+LTPPo	PilT ^{Pa}	DUDCm	
	DUTGm	PilTom	DUTGm	DUTGm	DUTGm			Pill ^{1a}	K58A	PilBom	
Sample	Tilted	nucleo-	1 mM	0.1 mM	1 mM			nucleo-	No	nucleo-	
Sampie	data	tide	ATP	ADP	ADP	0.1 mM	1 mM	tide	nucleo-	tide	
		added				ADP	ADP	added	tide	added	
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Selected	206422	0.500.5	50001	21	2 (01.1	,	14	10			
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Applied symmetry	maps		Ca		C		C.		C		
Applied B-fact	or $(Å^2)$	1	177		179		95		499	2	
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= 0.143, Å)	011 (1 0 0		4.1		4.0		4.4		/.8		
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	-	00	COOC		OCOCOC		CCCCC	С	CCOCCO		
Modeling softw	vare	Coot.	Phenix	(Coot, Phenix		Coot, Phe	nix	Coot, Phenix		
Atoms		1()426		10323		10416		11442		
Protein		1()426		10323		10416		11442		
ATP			0		0		186		0		
RMSD	8.	0	007		0.005		0.000	0.007			
Bond angle (°	A)	0.	06		1.05		1.05	0.007			
Ramachandara	<u>)</u> n	1	.00		1.05		1.05		1.4	5	
Favoured (%)			98		98		98		98		
Allowed		9	9.8		100		100		100)	
Clashscore			0.4		0.2		0.92		2.4	6	
Av. B-factor (Å	²)									_	
Protein			5.4		3.1		22.2		68.	8	
Ligand MolDrobity and	rot	0	-		-		24.6		- 1.0	2	
CC	107		50		0.03		0.78	1.03			
CCLiggend		U	-		-		0.57	0.42			
Deposited mar	os and coor	dinate files					0.07	-			
EMDB code		EMD	-20116]	EMD-20115		EMD-201	17	EMD-2	0114	
PDB code		60	DLL		60LK		60LM	60LJ			

*, As calculated using *MolProbity*¹⁹ implemented in *PHENIX*



Supplementary Figure 1 – Nomenclature used herein. Each PilT chain is composed of two domains: N2D and CTD. The packing unit is composed of the N2Dⁿ and CTDⁿ⁺¹. Two adjacent packing units contact one-another creating an interface between packing units. Six packing units organize in this manner to create a hexamer.

PilT ^{Gm}	', no r	ucleo	tide a	dded	139 mil.	- 100 miles	1041(#5%	550 pt.h	100000		ⁿ , 1 ml	M ATP	000000	000000	2100 pich	000000	000000	2003 profe	000000
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Supplementary Figure 2 – 2D class averages of PilB and PilT particles and their proposed conformation. The protein and nucleotide added are indicated above each list of 50 class averages. Class averages are sorted by the number of particles in each class. The qualitative assignment of the class average conformation is noted above each class average in white. A dotted outline of the conformation cartoon from Figure 4a is overlaid on the 2D class average for reference. Source data (without dotted outlines) are provided as a Source Data file. Class averages that were too blurry to identify the conformation are not annotated. Class averages that appear to represent tilted-views or side views are also labeled in white.



Supplementary Figure 3 – Local resolution (left), particle distribution (upper right), and Fourier shell correlation (FSC) curves (lower right). a, Locally sharpened PilT^{Gm} OOCOOC conformation map. b, Locally sharpened PilT^{Gm} OCOCOC conformation map. c, Locally sharpened PilT^{Gm} CCCCCC conformation map. d, Locally sharpened PilB^{Gm} CCOCCO conformation map.

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