

Supplemental data

Electrostatic Interactions between the CTX Phage Minor Coat Protein and the Bacterial Host Receptor TolA Drives the Pathogenic Conversion of *Vibrio cholerae*.

Laetitia Houot, Romain Navarro, Matthieu Nouailler, Denis Duché, Françoise Guerlesquin and Roland Llobes.

Table of content :

- S1- Expression of the wild-type pIN-pIII-N1^{CTX} His-tagged construct and individual cystein mutants.
 - S2- Expression and periplasmic transport of the pIII-N1 His-tagged constructs and point mutants of interest.
 - S3- List of *Vibrio cholerae* isolate genomes used to study TolA sequence conservation.
 - S4- Multiple TolAIII domain sequence alignment of representative members of the gammaproteobacteria family Vibrionaceae genus *Vibrio*
 - S5- Multiple TolAIII domain sequence alignment of representative members of the gammaproteobacteria family Vibrionaceae.
 - S6- Analysis of the TolAIII^{Vc}/pIII-N1^{CTX} complex interface using the open access tool 2P2I inspector
- Table S1 : Strains and plasmids used in the study
- Table S2 : Primers used in the study

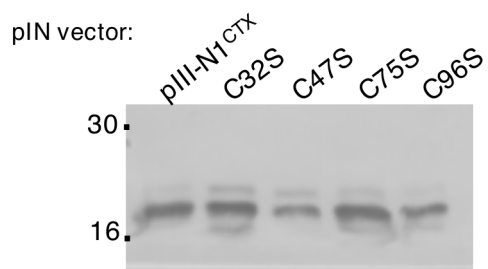


Fig. S1 : Expression of the wild-type pIN-pIII-N1^{CTX} His-tagged constructs and individual cysteine mutants. Western immunoblot of 0.2 OD units of whole-cell lysates of *E. coli* DH5 α strain carrying pIN-pIII-N1^{CTX}, or cysteine variants pIN-pIII-N1^{CTX} C32S, C47S, C75S and C96S, and probed with anti-His antibody. The molecular weight markers (in kDa) are indicated on the left.

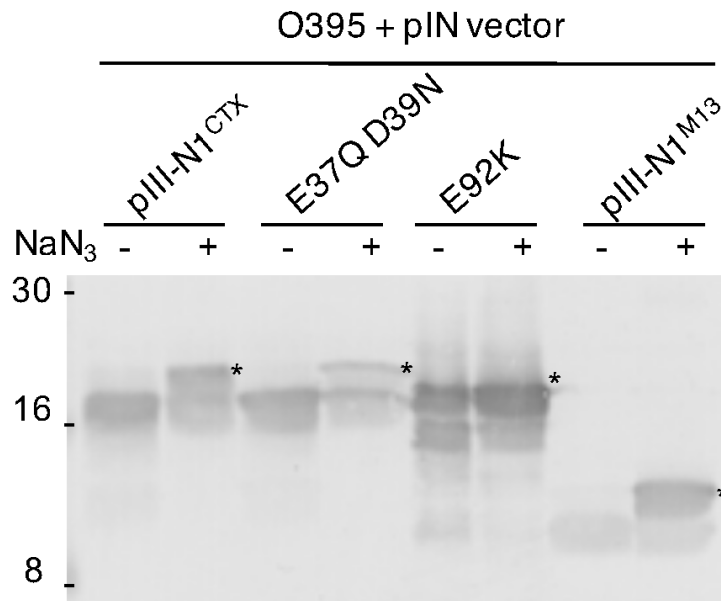


Fig. S2 : Expression and periplasmic transport of the pIII-N1 His-tagged constructs and point mutants of interest. *V. cholerae* O395 cells carrying pIN-pIII-N1^{CTX}, pIN-pIII-N1^{CTX} (E37Q D39N), pIN-pIII-N1^{CTX} (E92K) or pIN-pIII-N1^{M13} were grown to OD=0.3 in LB supplemented with MgCl₂, then incubated either with (+) or without (-) sodium azide (NaN₃ 1 mM) for 30 min at room temperature. Then, plasmid expression was induced by adding IPTG (100 μM) and incubating the cultures at 37°C for 2 hrs. A total of approximately 0.2 OD units of whole cell lysate were loaded on a 15% acrylamide gel SDS-PAGE and immunodetected using anti-His monoclonal antibody. The molecular weight markers (in kDa) are indicated on the left. Stars indicate the higher molecular weight bands corresponding to protein precursors that accumulate in the cytoplasm when the sec pathway is inhibited by NaN₃.

Supplemental data 3

Vibrio cholerae isolate

O395
1587
2012EL-1759
2740-80
2740-80
4260B
623-39
AM-19226
B33
BJG-01
BX 330286
C6706
CP1030(3)
CP1032(5)
CP1033(6)
CP1037(10)
CP1038(11)
CP1040(13)
CP1041(14)
CP1042(15)
CP1044(17)
CP1046(19)
CP1047(20)
CP1048(21)
CP1050(23)
HC-02A1
HC-02C1
HC-06A1
HC-17A1
HC-17A2
HC-19A1
HC-1A2
HC-20A2
HC-21A1
HC-22A1
HC-23A1
HC-28A1
HC-32A1
HC-33A2
HC-36A1
HC-37A1
HC-38A1
HC-39A1
HC-40A1
HC-41A1
HC-42A1
HC-43A1
HC-43B1
HC-46A1
HC-47A1
HC-48A1
HC-48B2
HC-49A2
HC-50A1
HC-50A2
HC-51A1
HC-52A1
HC-55A1
HC-55B2
HC-55C2
HC-56A1
HC-56A2
HC-57A1
HC-57A2
HC-59A1
HC-59B1

TolA protein ID

AAG17041.1 TolA [Vibrio cholerae]
KNH58248.1 TolA protein [Vibrio cholerae]
KEH04570.1 TolA [Vibrio cholerae]
KNA61815.1 TolA protein [Vibrio cholerae]
ANR87163.1 protein TolA [Vibrio cholerae]
ELP50441.1 TolA protein [Vibrio cholerae]
KNH55594.1 TolA protein [Vibrio cholerae]
KNA56762.1 TolA protein [Vibrio cholerae]
EEO16601.1 TolA protein [Vibrio cholerae]
EGS68611.1 protein TolA [Vibrio cholerae]
EEO20492.1 TolA protein [Vibrio cholerae]
OFJ22580.1 protein TolA [Vibrio cholerae]
EJH83233.1 protein TolA [Vibrio cholerae]
EJH27184.1 protein TolA [Vibrio cholerae]
EKK81010.1 protein TolA [Vibrio cholerae]
EKG68196.1 protein TolA [Vibrio cholerae]
EJH29429.1 protein TolA [Vibrio cholerae]
EKG69121.1 protein TolA [Vibrio cholerae]
EJH28074.1 protein TolA [Vibrio cholerae]
EJH38367.1 protein TolA [Vibrio cholerae]
EKG78113.1 protein TolA [Vibrio cholerae]
EJH39131.1 protein TolA [Vibrio cholerae]
EJH85493.1 protein TolA [Vibrio cholerae]
EJH42408.1 protein TolA [Vibrio cholerae]
EKG79384.1 protein TolA [Vibrio cholerae]
EGS61263.1 protein TolA [Vibrio cholerae]
EKL92294.1 protein TolA [Vibrio cholerae]
EHH71180.1 protein TolA [Vibrio cholerae]
EKK96962.1 protein TolA [Vibrio cholerae]
EKL86114.1 protein TolA [Vibrio cholerae]
EHH76472.1 protein TolA [Vibrio cholerae]
EKK93092.1 protein TolA [Vibrio cholerae]
EJH49963.1 protein TolA [Vibrio cholerae]
EHH72034.1 protein TolA [Vibrio cholerae]
EHH81683.1 protein TolA [Vibrio cholerae]
EHH83325.1 protein TolA [Vibrio cholerae]
EHH86659.1 protein TolA [Vibrio cholerae]
EHH93028.1 protein TolA [Vibrio cholerae]
EHH98450.1 protein TolA [Vibrio cholerae]
ERP67718.1 protein TolA [Vibrio cholerae]
EKL85345.1 protein TolA [Vibrio cholerae]
EGS70402.1 protein TolA [Vibrio cholerae]
EKG45469.1 protein TolA [Vibrio cholerae]
EGS48190.1 protein TolA [Vibrio cholerae]
EKG51082.1 protein TolA [Vibrio cholerae]
EJH72841.1 protein TolA [Vibrio cholerae]
EHH97458.1 protein TolA [Vibrio cholerae]
EJH51131.1 protein TolA [Vibrio cholerae]
EJH53444.1 protein TolA [Vibrio cholerae]
EJH80761.1 protein TolA [Vibrio cholerae]
EGS47519.1 protein TolA [Vibrio cholerae]
EHI03882.1 protein TolA [Vibrio cholerae]
EGQ97343.1 protein TolA [Vibrio cholerae]
EKG50642.1 protein TolA [Vibrio cholerae]
EKL06240.1 protein TolA [Vibrio cholerae]
EKG90430.1 protein TolA [Vibrio cholerae]
EKG55816.1 protein TolA [Vibrio cholerae]
EKG61388.1 protein TolA [Vibrio cholerae]
EKL99738.1 protein TolA [Vibrio cholerae]
EKL06804.1 protein TolA [Vibrio cholerae]
EKG60702.1 protein TolA [Vibrio cholerae]
EJH69589.1 protein TolA [Vibrio cholerae]
EKG69728.1 protein TolA [Vibrio cholerae]
EJH70213.1 protein TolA [Vibrio cholerae]
EKL12669.1 protein TolA [Vibrio cholerae]
EKM06391.1 protein TolA [Vibrio cholerae]

HC-60A1	EKL12763.1	protein	TolA	[Vibrio cholerae]
HC-61A1	EHT05342.1	protein	TolA	[Vibrio cholerae]
HC-61A2	EKL20125.1	protein	TolA	[Vibrio cholerae]
HC-62A1	EKL26204.1	protein	TolA	[Vibrio cholerae]
HC-62B1	EKM09587.1	protein	TolA	[Vibrio cholerae]
HC-64A1	ELS73696.1	protein	TolA	[Vibrio cholerae]
HC-65A1	ELS81445.1	protein	TolA	[Vibrio cholerae]
HC-67A1	ELS88637.1	protein	TolA	[Vibrio cholerae]
HC-68A1	ELS96209.1	protein	TolA	[Vibrio cholerae]
HC-69A1	EKM17783.1	protein	TolA	[Vibrio cholerae]
HC-70A1	EGS47438.1	protein	TolA	[Vibrio cholerae]
HC-71A1	ELT03739.1	protein	TolA	[Vibrio cholerae]
HC-72A2	ELT09806.1	protein	TolA	[Vibrio cholerae]
HC-77A1	EKL25291.1	protein	TolA	[Vibrio cholerae]
HC-78A1	ELT17500.1	protein	TolA	[Vibrio cholerae]
HC-7A1	ELT24567.1	protein	TolA	[Vibrio cholerae]
HC-80A1	ELT32189.1	protein	TolA	[Vibrio cholerae]
HC-81A1	ELT39883.1	protein	TolA	[Vibrio cholerae]
HC-81A2	EKG88838.1	protein	TolA	[Vibrio cholerae]
HCUF01	EGQ98317.1	protein	TolA	[Vibrio cholerae]
HE-25	EJH62632.1	protein	TolA	[Vibrio cholerae]
HFU-02	EGS62215.1	protein	TolA	[Vibrio cholerae]
IRS 101	EET92627.1	TolA protein		[Vibrio cholerae]
M66-2	ACP06066.1	tolA protein		[Vibrio cholerae]
MAK 757	EFH78507.1	TolA		[Vibrio cholerae]
MJ-1236	ACQ60705.1	TolA protein		[Vibrio cholerae]
MO10	EET23042.1	tolA protein		[Vibrio cholerae]
MS6	BAP03257.1	TolA protein		[Vibrio cholerae]
MZO-3	KNA50641.1	TolA protein		[Vibrio cholerae]
O1 biovar El tor Str. L-3226	EYC49350.1	TolA		[Vibrio cholerae]
O1 biovar El Tor str. N16961	AAF94985.1	tolA protein		[Vibrio cholerae]
O1 str. 116059	EMP83962.1	protein	TolA	[Vibrio cholerae]
O1 str. 2010EL-1786	AET26930.1	TolA protein		[Vibrio cholerae]
O1 str. 95412	EMP97232.1	protein	TolA	[Vibrio cholerae]
O1 str. AG-7404	EMP95143.1	protein	TolA	[Vibrio cholerae]
O1 str. AG-8040	EMQ02788.1	protein	TolA	[Vibrio cholerae]
O1 str. EC-0009	EMQ04249.1	protein	TolA	[Vibrio cholerae]
O1 str. EC-0012	EMQ11023.1	protein	TolA	[Vibrio cholerae]
O1 str. EC-0027	EMQ10848.1	protein	TolA	[Vibrio cholerae]
O1 str. EC-0051	EMQ15676.1	protein	TolA	[Vibrio cholerae]
O1 str. EDC-020	EMQ21633.1	protein	TolA	[Vibrio cholerae]
O1 str. EDC-022	EMQ26604.1	protein	TolA	[Vibrio cholerae]
O1 str. EM-1536	EMQ25667.1	protein	TolA	[Vibrio cholerae]
O1 str. EM-1546	EMQ31601.1	protein	TolA	[Vibrio cholerae]
O1 str. EM-1626	EMQ37289.1	protein	TolA	[Vibrio cholerae]
O1 str. EM-1676A	EMQ51732.1	protein	TolA	[Vibrio cholerae]
O1 str. EM-1727	EMQ50741.1	protein	TolA	[Vibrio cholerae]
O1 str. Inaba G4222	EMB02008.1	TolA protein		[Vibrio cholerae]
O1 str. Nep-21106	EMQ62444.1	protein	TolA	[Vibrio cholerae]
O1 str. Nep-21113	EMQ42466.1	protein	TolA	[Vibrio cholerae]
O1 str. NHCC-004A	EMQ53021.1	protein	TolA	[Vibrio cholerae]
O1 str. NHCC-006C	EMQ38536.1	protein	TolA	[Vibrio cholerae]
O1 str. NHCC-008D	EMQ65782.1	protein	TolA	[Vibrio cholerae]
O1 str. NHCC-010F	EMQ67729.1	protein	TolA	[Vibrio cholerae]
O1 str. PCS-022	EWM32085.1	protein	TolA	[Vibrio cholerae]
O1 str. PCS-023	EMQ47243.1	protein	TolA	[Vibrio cholerae]
PS15	EKY33057.1	TolA protein		[Vibrio cholerae]
RC385	EFH75783.1	tolA protein		[Vibrio cholerae]
RC9	EEO08498.1	TolA protein		[Vibrio cholerae]
TMA 21	EEO12881.1	TolA protein		[Vibrio cholerae]
V51	KNA58939.1	TolA protein		[Vibrio cholerae]
V52	KNH50562.1	TolA protein		[Vibrio cholerae]

Fig. S3 : List of *Vibrio cholerae* isolate genomes used to study TolA sequence conservation. The TolA protein sequences retrieved from the listed 128 *Vibrio cholerae* isolates present 100% identity.

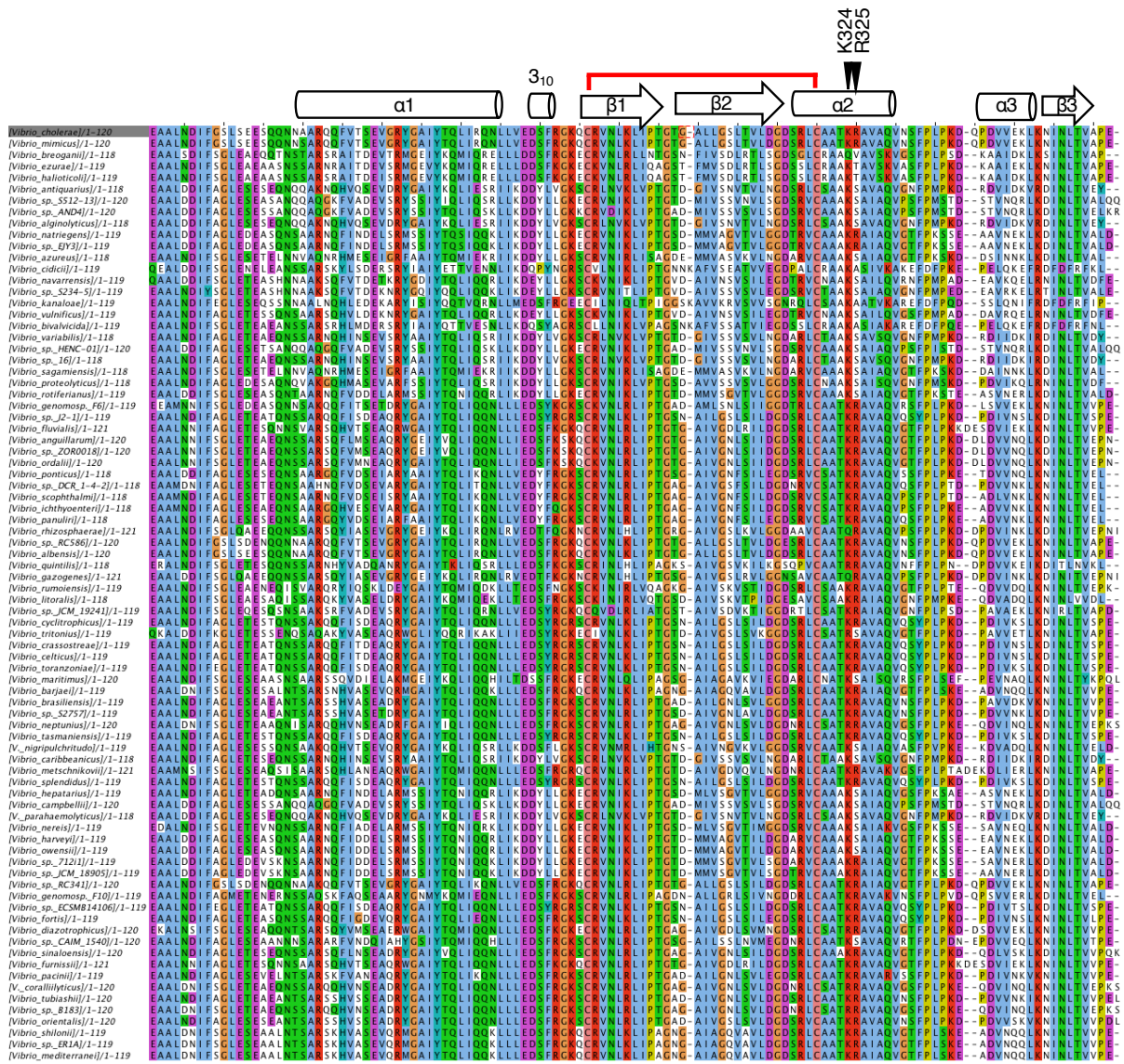


Fig. S4: Multiple TolAIII domain sequence alignment of representative members of the gammaproteobacteria family Vibrionaceae genus *Vibrio*. The depicted secondary-structure elements correspond to the crystal structure of *V. cholerae* TolAIII (PDB: 4G7X) with β -strands as arrows and α -helices as a cylinders. Positions are colored according to the Clustal color scheme: cysteines in pink, glycines in orange and prolines in yellow; other positions are colored according to conservation of chemical properties: hydrophobic in blue, aromatic in cyan, polar negative in purple, polar positive in red, and polar neutral in green.

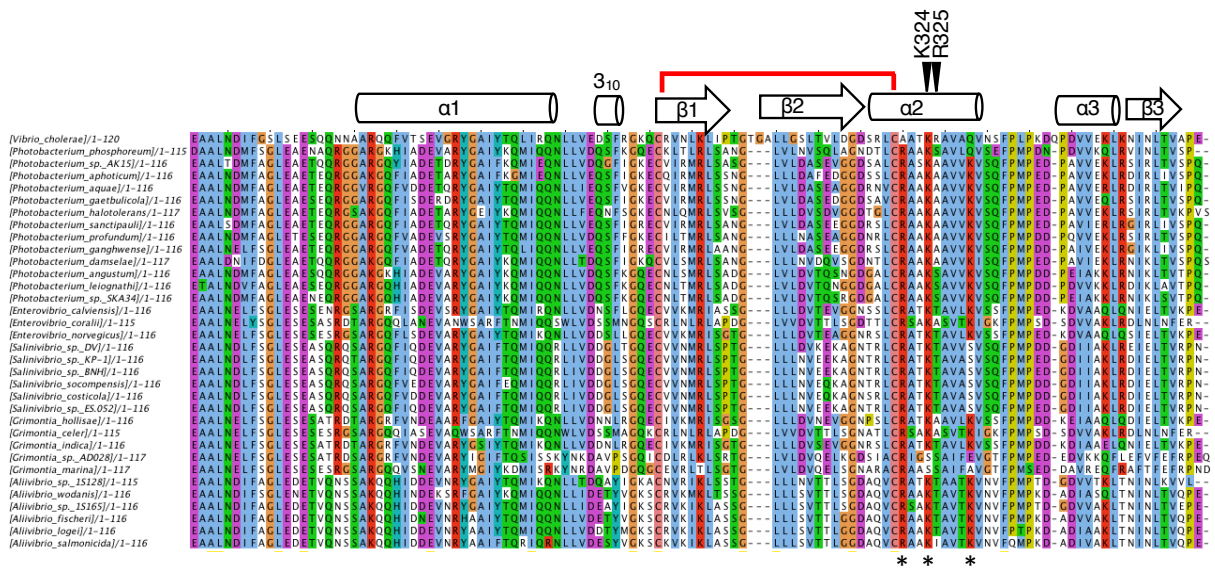


Fig. S5: Multiple TolAIII domain sequence alignment of representative members of the gammaproteobacteria family Vibrionaceae. The depicted secondary-structure elements correspond to the crystal structure of *V. cholerae* TolAIII (PDB: 4G7X) with β -strands as arrows and α -helices as a cylinders. Positions are colored according to the Clustal color scheme: cysteines in pink, glycines in orange and prolines in yellow; other positions are colored according to conservation of chemical properties: hydrophobic in blue, aromatic in cyan, polar negative in purple, polar positive in red, and polar neutral in green. Stars indicate the positive charged residues present on the same side of the $\alpha 2$ -helix.

	Atom no.	Atom name	Res name	Res no.	Chain		Atom no.	Res name	Res name	Res no.	Chain
1.	102	O	THR	10	A	<-->	2257	O	LEU	310	B
2.	102	O	THR	10	A	<-->	2278	CG2	THR	311	B
3.	102	O	THR	10	A	<-->	2275	C	THR	311	B
4.	102	O	THR	10	A	<-->	2274	CA	THR	311	B
5.	105	OG1	THR	10	A	<-->	2278	CG2	THR	311	B
6.	102	O	THR	10	A	<-->	2287	N	VAL	312	B
7.	105	OG1	THR	10	A	<-->	2290	O	VAL	312	B
8.	103	CB	THR	10	A	<-->	2290	O	VAL	312	B
9.	104	CG2	THR	10	A	<-->	2290	O	VAL	312	B
10.	118	CG2	THR	11	A	<-->	2246	CB	SER	309	B
11.	115	C	THR	11	A	<-->	2257	O	LEU	310	B
12.	118	CG2	THR	11	A	<-->	2257	O	LEU	310	B
13.	114	CA	THR	11	A	<-->	2257	O	LEU	310	B
14.	119	OG1	THR	11	A	<-->	2279	OG1	THR	311	B
15.	119	OG1	THR	11	A	<-->	2278	CG2	THR	311	B
16.	119	OG1	THR	11	A	<-->	2277	CB	THR	311	B
17.	118	CG2	THR	11	A	<-->	2279	OG1	THR	311	B
18.	117	CB	THR	11	A	<-->	2279	OG1	THR	311	B
19.	114	CA	THR	11	A	<-->	2279	OG1	THR	311	B
20.	130	O	SER	12	A	<-->	2246	CB	SER	309	B
21.	130	O	SER	12	A	<-->	2243	CA	SER	309	B
22.	130	O	SER	12	A	<-->	2244	C	SER	309	B
23.	130	O	SER	12	A	<-->	2257	O	LEU	310	B
24.	130	O	SER	12	A	<-->	2254	N	LEU	310	B
25.	131	CB	SER	12	A	<-->	2257	O	LEU	310	B
26.	128	CA	SER	12	A	<-->	2257	O	LEU	310	B
27.	127	N	SER	12	A	<-->	2257	O	LEU	310	B
28.	127	N	SER	12	A	<-->	2256	C	LEU	310	B
29.	132	OG	SER	12	A	<-->	2463	NZ	LYS	324	B
30.	132	OG	SER	12	A	<-->	2462	CE	LYS	324	B
31.	140	C	HIS	13	A	<-->	2238	O	GLY	308	B
32.	139	CA	HIS	13	A	<-->	2238	O	GLY	308	B
33.	163	OE1	GLN	14	A	<-->	2220	CB	LEU	307	B
34.	163	OE1	GLN	14	A	<-->	2223	CD2	LEU	307	B
35.	162	NE2	GLN	14	A	<-->	2220	CB	LEU	307	B
36.	161	CD	GLN	14	A	<-->	2220	CB	LEU	307	B
37.	162	NE2	GLN	14	A	<-->	2219	O	LEU	307	B
38.	161	CD	GLN	14	A	<-->	2219	O	LEU	307	B
39.	160	CG	GLN	14	A	<-->	2219	O	LEU	307	B
40.	162	NE2	GLN	14	A	<-->	2216	N	LEU	307	B
41.	159	CB	GLN	14	A	<-->	2223	CD2	LEU	307	B
42.	159	CB	GLN	14	A	<-->	2219	O	LEU	307	B
43.	159	CB	GLN	14	A	<-->	2238	O	GLY	308	B
44.	155	N	GLN	14	A	<-->	2237	C	GLY	308	B
45.	155	N	GLN	14	A	<-->	2238	O	GLY	308	B
46.	158	O	GLN	14	A	<-->	2238	O	GLY	308	B
47.	156	CA	GLN	14	A	<-->	2238	O	GLY	308	B
48.	510	OE1	GLU	37	A	<-->	2463	NZ	LYS	324	B
49.	510	OE1	GLU	37	A	<-->	2462	CE	LYS	324	B
50.	509	CD	GLU	37	A	<-->	2463	NZ	LYS	324	B
51.	547	OD2	ASP	39	A	<-->	2481	CB	ARG	325	B

52.	547	OD2	ASP	39	A	<-->	2486	NH1	ARG	325	B
53.	547	OD2	ASP	39	A	<-->	2485	CZ	ARG	325	B
54.	547	OD2	ASP	39	A	<-->	2484	NE	ARG	325	B
55.	547	OD2	ASP	39	A	<-->	2483	CD	ARG	325	B
56.	546	OD1	ASP	39	A	<-->	2486	NH1	ARG	325	B
57.	546	OD1	ASP	39	A	<-->	2485	CZ	ARG	325	B
58.	546	OD1	ASP	39	A	<-->	2484	NE	ARG	325	B
59.	545	CG	ASP	39	A	<-->	2486	NH1	ARG	325	B
60.	545	CG	ASP	39	A	<-->	2485	CZ	ARG	325	B
61.	545	CG	ASP	39	A	<-->	2484	NE	ARG	325	B
62.	568	CB	TYR	41	A	<-->	2485	CZ	ARG	325	B
63.	568	CB	TYR	41	A	<-->	2487	NH2	ARG	325	B
64.	587	C	GLY	42	A	<-->	2486	NH1	ARG	325	B
65.	588	O	GLY	42	A	<-->	2486	NH1	ARG	325	B
66.	595	O	PHE	43	A	<-->	2486	NH1	ARG	325	B
67.	594	C	PHE	43	A	<-->	2486	NH1	ARG	325	B
68.	613	CA	VAL	44	A	<-->	2486	NH1	ARG	325	B
69.	618	CG2	VAL	44	A	<-->	2486	NH1	ARG	325	B
70.	612	N	VAL	44	A	<-->	2486	NH1	ARG	325	B
71.	617	CG1	VAL	44	A	<-->	2531	CB	ALA	328	B
72.	681	CD2	LEU	48	A	<-->	2577	OD1	ASN	331	B
73.	739	ND2	ASN	52	A	<-->	2150	CG2	THR	301	B
74.	740	OD1	ASN	52	A	<-->	2204	CD2	LEU	306	B
75.	777	OH	TYR	55	A	<-->	2573	O	ASN	331	B
76.	805	NZ	LYS	57	A	<-->	2530	O	ALA	328	B
77.	805	NZ	LYS	57	A	<-->	2529	C	ALA	328	B
78.	804	CE	LYS	57	A	<-->	2530	O	ALA	328	B
79.	805	NZ	LYS	57	A	<-->	2556	C	VAL	330	B
80.	805	NZ	LYS	57	A	<-->	2557	O	VAL	330	B
81.	834	CD2	LEU	59	A	<-->	2223	CD2	LEU	307	B
82.	833	CD1	LEU	59	A	<-->	2458	O	LYS	324	B
83.	833	CD1	LEU	59	A	<-->	2531	CB	ALA	328	B
84.	832	CG	LEU	59	A	<-->	2531	CB	ALA	328	B
85.	1344	OE1	GLU	92	A	<-->	2823	CE	LYS	347	B
86.	1344	OE1	GLU	92	A	<-->	2824	NZ	LYS	347	B
87.	1342	CG	GLU	92	A	<-->	2824	NZ	LYS	347	B
88.	1343	CD	GLU	92	A	<-->	2824	NZ	LYS	347	B

Fig. S6 : Analysis of the TolAIII^{Vc}/pIII-N1^{CTX} complex interface using the open access tool 2P2I inspector. The following parameters were applied : two atoms are considered to be in contact across the interface if they are at a distance smaller than 4 Å. Non-bonded contacts between pIII-N1^{CTX} (Chain A) and TolAIII^{Vc} (Chain B) atoms are listed. Contacts engaged by TolAIII residue K324 are colored in red and residue R325 are colored in green.

Table S1 : Strains and plasmids used in the study.

Strains and plasmids	Genotype or description	Source/References
<i>E. coli</i>		
DH5α	F- Φ80/ <i>lacZ</i> ΔM15 Δ(<i>lacZYA-argF</i>) U169 <i>recA1 endA1 hsdR17</i> (rK-, mK+) <i>phoA supE44 λ- thi-1 gyrA96 relA1</i>	Laboratory collection
CC118λpir	Δ(<i>ara-leu</i>), <i>araD</i> , Δ <i>lacX74</i> , <i>galE</i> , <i>galK</i> , <i>phoA20</i> , <i>thi-1</i> , <i>rpsE</i> , <i>rpoB</i> , <i>argE</i> (Am), <i>recA</i> , λpir phage lysogen	Laboratory collection
SM10λpir	KmR, <i>thi-1</i> , <i>thr</i> , <i>leu</i> , <i>tonA</i> , <i>lacY</i> , <i>supE</i> , <i>recA::RP4-2-Tc::Mu</i> , <i>pir</i>	Laboratory collection
BTH101	F-, <i>cya-99</i> , <i>araD139</i> , <i>galE15</i> , <i>galK16</i> , <i>rpsL1</i> (Str ^R), <i>hsdR2</i> , <i>mcrA1</i> , <i>mcrB1</i> .	Ref. (29)
SHuffle T7	F' <i>lac</i> , <i>pro</i> , <i>lacI^Q</i> / Δ(<i>ara-leu</i>)7697 <i>araD139 fhuA2 lacZ::T7 gene1</i> Δ(<i>phoA</i>) <i>PvuII phoR ahpC* galE</i> (or <i>U galK</i> <i>λatt::pNEB3-r1-cDsbC</i> (Spec ^R , <i>lacI^Q</i>) Δ <i>trxB rpsL150</i> (Str ^R) Δ <i>gor</i> Δ(<i>malF</i>)3	NEB
Oxi-BTH	SHuffle T7 <i>cya</i> ^o	This study
W3110	F- lambda- IN(rrnD-rrnE)1 <i>rph-1</i>	Laboratory collection
<i>V. cholerae</i>		
O395	O1 classical Ogawa; SmR	Gift from Dr. FX. Barre
O395 <i>tolA</i> Δ(41-421)	O395 bearing a deletion of <i>tolA</i> sequence, resulting in a protein missing amino acid 41 to 421; SmR ApR	This study
N16961	O1 El Tor; SmR	Gift from Dr. FX. Barre
BS2 pCTX-cm	N16961 <i>dif1</i> - carrying the replicative form of CTX-Cm phage; SmR CmR	Ref. (71)
<i>V. anguillarum</i>	ATCC19264	Gift from Dr. D. Destoumieux-Gazon
<i>V. fischeri</i>	ES114	Gift from Dr. MS. Aschtgen
<i>V. harveyi</i>	LMG11659	Gift from Dr. MS. Aschtgen
<i>V. tasmaniensis</i>	LMG20012T	Gift from Dr. D. Destoumieux-Gazon
<i>V. alginolyticus</i>	ATCC 17749	Gift from Dr. LF. Wu
Plasmids used in rescue experiments		
pBAD18km	Arabinose-inducible plasmid, KanR	Ref. (70)
pBAD- <i>tolA</i> ^{Vc}	pBAD18km carrying a consensus SD and <i>V. cholerae tolA</i> gene, KmR	This study
pBAD- <i>tolA</i> ^{Vc} (K324E)	pBAD- <i>tolA</i> ^{Vc} bearing a K-to-E substitution at position 324, KmR	This study
pBAD- <i>tolA</i> ^{Vc} (R325D)	pBAD- <i>tolA</i> ^{Vc} bearing a R-to-D substitution at position 325, KmR	This study
pBAD- <i>tolA</i> ^{Vc} (K324E-R325D)	pBAD- <i>tolA</i> ^{Vc} bearing K324E and R325D substitutions, KmR	This study
pBAD- <i>tolA</i> ^{Vc} (K347E)	pBAD- <i>tolA</i> ^{Vc} bearing a K-to-E substitution at position 347, KmR	This study
Plasmids used for periplasmic expression		
pIN	pIN-III-ompA2 vector, <i>ompA</i> signal sequence followed by a multi cloning site, ApR	Laboratory collection
pIN-pIII-N1 ^{CTX}	pIN vector carrying fragment of CTX PIII encoding gene (residues 15 to 138), N-terminal Strep Tag epitope, C-terminal 6-His epitope, ApR	This study
pIN-pIII-N1 ^{CTX} (E37Q-D39N)	pIN-pIII-N1 ^{CTX} bearing a E-to-Q mutation at position 37 and D-to-N mutation at position 39, ApR	This study
pIN-pIII-N1 ^{CTX} (E92K)	pIN-pIII-N1 ^{CTX} bearing a E-to-K mutation at position 92, ApR	This study
pIN-pIII-N1 ^{CTX} (C32S)	pIN-pIII-N1 ^{CTX} bearing a C-to-S mutation at position 32, ApR	This study

pIN-pIII-N1 ^{CTX} (C47S)	pIN-PIII-N1CTX bearing a C-to-S mutation at position 47, ApR	This study
pIN-pIII-N1 ^{CTX} (C75S)	pIN-PIII-N1CTX bearing a C-to-S mutation at position 75, ApR	This study
pIN-pIII-N1 ^{CTX} (C96S)	pIN-PIII-N1CTX bearing C-to-S mutation at position 96, ApR	This study
pIN-pIII-N1 ^{M13}	pINIII-ompA2 vector carrying a fragment of M13 PIII encoding gene, N-terminal Strep Tag epitope, C-terminal 6-His epitope, ApR	Ref. (10)
Plasmids used in Bacterial 2-hybrid experiments		
pUT18c	BACTH expression vector, ApR	Ref. (29)
pUT18-TolAIII ^{Ec}	<i>E. coli tolA</i> sequence (residues 292 to 421) cloned downstream T18 into pUT18c, ApR	This study
pUT18-TolAIII ^{Vc}	<i>V. cholerae tolA</i> sequence (residues 239 to 356) cloned downstream T18 into pUT18c, ApR	This study
pUT18-TolAIII ^{Vc} (C292S)	pUT18-TolA3Vc bearing a C-to-S mutation at position 292	This study
pUT18-TolAIII ^{Vc} (K324E-R325D)	pUT18-TolA3Vc bearing K-to-E and R-to-D mutations at positions 324 and 325, respectively	This study
pUT18-TolAIII ^{Vc} (K324E)	pUT18-TolA3Vc bearing K-to-E mutations at positions 324	This study
pUT18-TolAIII ^{Vc} (R325D)	pUT18-TolA3Vc bearing R-to-D mutations at positions 325	This study
pUT18-TolAIII ^{Vc} (K347E)	pUT18-TolA3Vc bearing a K-to-E mutation at position 347	This study
pUT18-TolAIII <i>V. alginolyticus</i>	<i>V. alginolyticus tolA</i> sequence (residues 239 to 356) cloned downstream T18 into pUT18c, ApR	This study
pUT18-TolAIII <i>V. anguillarum</i>	<i>V. anguillarum tolA</i> sequence (residues 239 to 356) cloned downstream T18 into pUT18c, ApR	This study
pUT18-TolAIII <i>V. fischeri</i>	<i>V. fischeri tolA</i> sequence (residues 233 to 346) cloned downstream T18 into pUT18c, ApR	This study
pUT18-TolAIII <i>V. tasmaniensis</i>	<i>V. tasmaniensis tolA</i> sequence (residues 239 to 356) cloned downstream T18 into pUT18c, ApR	This study
pUT18-TolAIII <i>V. harveyi</i>	<i>V. harveyi tolA</i> sequence (residues 239 to 356) cloned downstream T18 into pUT18c, ApR	This study
pKT25	BACTH expression vector, KmR	Ref. (29)
pKT25-pIII-N1 ^{M13}	M13 pIII sequence (residues 20 to 89) cloned downstream T25 into pKT25	This study
pKT25-pIII-N1 ^{CTX}	CTX pIII sequence (residues 15 to 136) cloned downstream T25 into pKT25	This study
pKT25 pIII-N2 ^{CTX}	CTX pIII sequence (residues 130 to 266) cloned downstream T25 into pKT25	This study
pKT25-pIII-N1N2 ^{CTX}	CTX pIII sequence (residues 15 to 266) cloned downstream T25 into pKT25	This study
pKT25-colAN	colicin A sequence (residues 1 to 178) cloned downstream T25 into pKT25	Gift from Dr. E. Bouveret
pKT25-pIII-N1 ^{CTX} (C32S)	pKT25-PIII-N1 ^{CTX} bearing a C-to-S mutation at position 32	This study
pKT25-pIII-N1 ^{CTX} (C47S)	pKT25-PIII-N1 ^{CTX} bearing a C-to-S mutation at position 47	This study
pKT25-pIII-N1 ^{CTX} (C75S)	pKT25-PIII-N1 ^{CTX} bearing a C-to-S mutation at position 85	This study
pKT25-pIII-N1 ^{CTX} (C96S)	pKT25-PIII-N1 ^{CTX} bearing a C-to-S mutation at position 90	This study
pKT25-pIII-N1 ^{CTX} (E37Q-D39N)	pKT25-PIII-N1 ^{CTX} bearing E-to-Q and D-to-N mutations at position 37 and 39, respectively	This study
pKT25-pIII-N1 ^{CTX} (E37K-D39R)	pKT25-PIII-N1 ^{CTX} bearing E-to-K and D-to-R mutations at position 37 and 39, respectively	This study
pKT25-pIII-N1 ^{CTX} (E92K)	pKT25-PIII-N1 ^{CTX} bearing a E-to-K mutation at position 92	This study

Table S2 : Primers used in the study

PRIMERS	NAME	SEQUENCES
BACTH experiments		
pUT18-TolAIII ^{Vc}	oLH173	GCAGTGGAACGCCACTGCAGGGCCTTGAATGATATTTTTGGC
	oLH174	GATGAATTCGAGCTCGGTACCTCAGGTGCTACGGTTAAATTAATATTC
pUT18-TolAIII ^{Ec}	oLH207	GCAGTGGAACGCCACTGCAGCGCAGAGGCAGATGATATTTTTCG
	oLH208	GATGAATTCGAGCTCGGTACCACGGTTTGAAGTCCAATGGCGCG
pKT25-pIII-N1 ^{CTX}	oLH175	CGCACGCGGGGGCTGCAGCTCCATCGGTAACGGCTTCC
	oLH176	CTTAGTACTTAGGTACCGCAGACTTAAACGAGCTG
pKT25 pIII-N2 ^{CTX}	oLH226	CGCACGCGGGGGCTGCAGCTGGGCTGAGCAGCTCGTTTAAGTC
	oLH211	CTTAGTACTTAGGTACCCTGCTGCTATCGGGTGGCGTGGGATT
pKT25-pIII-N1 ^{M13}	oLH209	CGCACGCGGGGGCTGCAGCTGCTGAAACTGTTGAAAGTTGTTTAGC
	oLH210	CTTAGTACTTAGGTACCGAGCCACCACCCTCATTTTCAGGG
pUT18-TolAIII <i>V. tasmaniensis</i>	oLH363	GCAGTGGAACGCCACTGCAGAGCGGCATTGAACGATATTTTTGC
	oLH364	GATGAATTCGAGCTCGGTACCTCTGGTGATACAGTTAAGTTAATG
pUT18-TolAIII <i>V. fischeri</i>	oLH367	GCAGTGGAACGCCACTGCAGAGCATTAAATGATATCTTTGCAG
	oLH368	GATGAATTCGAGCTCGGTACTTCTGGTTGAACGGTAAGTTTATATTC
pUT18-TolAIII <i>V. anguillarum</i>	oLH369	GCAGTGGAACGCCACTGCAGGGCGCTGAATAACATTTTTTCTGG
	oLH370	GATGAATTCGAGCTCGGTACATTTGGTTCTACAGTTAAATTAATGTC
pUT18-TolAIII <i>V. alginolyticus</i>	oLH381	GCAGTGGAACGCCACTGCAGAGAAGCAGCTGAGCGTGCCGAGCGTGA
	oLH382	GATGAATTCGAGCTCGGTACGTCCAAAGCAACAGTTATGTTGATGTC
pUT18-TolAIII <i>V. harveyi</i>	oLH379	GCAGTGGAACGCCACTGCAGAGAGCGTCTTGAGCGATTAGAGCG
	oLH380	GATGAATTCGAGCTCGGTACATAATCAACCGTTAAGTTAACATCAG
Complementation construct		
pBAD-tolA ^{Vc}	oLH373	GTTTTTTTGGGCTAGCGAATTCGAAGGAGATATACATACCCATGAAAG AGAATAAGTCCAGAAAAAGTAATGATGC
	oLH374	TGCCTGCAGGTCGACTCTAGATTATTCAGGTGCTACGGTTAAATTAAT ATTCTTTAG
Periplasmic expression construct		
pIN-pIII-N1 ^{CTX}	oLH7	TAGAATTCTGGTCTCATCCTCAATTTGAAAAACCATCGGTAACGGCTT CCGCCATCAATTG
	oLH8	TAGGATCCTCAATGGTGATGGTGATGATGCGCAGACTTAAACGAGCTG CTCAG
Quick Change mutagenesis		
TolAIII ^{Vc} (C292S)	oLH105	GTTTTTCGAGGAAAGCAGTCTAGGGTAAACCTAAAGCTTATTCCTACTG
	oLH106	GCTTTAGGTTTACCCTAGACTGCTTTCCTCGAAAAGTGTCTTCC
TolAIII ^{Vc} (K324E-R325D)	oLH109	TTTATGTGCAGCAACCGAAGACGCTGTTGCCCAAGTGAATAG
	oLH110	CTTGGGCAACAGCGTCTTCGGTTGCTGCACATAAACGACTG
TolAIII ^{Vc} (K324E)	oLH375	TTTATGTGCAGCAACCGAAGACGCTGTTGCCCAAGTGAATAG
	oLH376	CTTGGGCAACAGCGTCTTCGGTTGCTGCACATAAACGACTG
TolAIII ^{Vc} (R325D)	oLH377	TTTATGTGCAGCAACCGAAGACGCTGTTGCCCAAGTGAATAG
	oLH378	CTTGGGCAACAGCGTCTTTCGGTTGCTGCACATAAACGACTG
TolAIII ^{Vc} (K347E)	oLH234	GTTGTGGAAAACTAGAGAATATTAATTTAACCG
	oLH235	CGGTTAAATTAATATTTCTCTAGTTTTTCCACAAC

pIII-N1 ^{CTX} (C32S)	oLH212	GTTATTTGATGGCTCCATGCTTGAT
	oLH213	ATCAAGCATGGAGCCATCAAATAAC
pIII-N1 ^{CTX} (C47S)	oLH214	GTTTGGTCTTCTCTCTCAAATGAAAATG
	oLH215	CATTTTCATTTGAGAGAGAAGACCAAAC
pIII-N1 ^{CTX} (C75S)	oLH216	GCATCTGCAGAGCGCTCCATTTATCC
	oLH217	GGATAAATGGAGCGCTCTGCAGATGC
pIII-N1 ^{CTX} (C96S)	oLH224	GCATTTATCCTTATTCCCCTGAGGGG
	oLH225	CCCCTCAGGGGAATAAGGATAAATGC
pIII-N1 ^{CTX} (E37Q-D39N)	oLH113	GGCTGCATGCTTGATATTCAAAAAAATGACTATGGTTTTGTTGGTC
	oLH114	GACCAAACAAAACCATAGTCATTTTTTTGAATATCAAGCATGCAGCC
pIII-N1 ^{CTX} (E37K-D39R)	oLH220	GGCTGCATGCTTGATATTAAAAAACGTGACTATGGTTTTGTTGGTC
	oLH221	GACCAAACAAAACCATAGTCACGTTTTTTAATATCAAGCATGCAGCC
pIII-N1 ^{CTX} (E92K)	oLH222	ATCCTTATTGCCCTAAGGGGAAGAGTGC
	oLH223	GCACTCTTCCCCCTTAGGGCAATAAGGAT
ToIAIII <i>V. harveyi</i> (S325R)	oLH388	CTTGATTAAGTGCCTCTTTGCTGC
	oLH389	GTATGTGCGGCTGCGAAGAGAGCGATTGCGCAGGTAG