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Supplementary Table 1 | BinA Structure-Function Summary Table

Domain and Subdomain boundaries			
M1-F10[18]	**N-terminal pro-peptide**		In the native structure (pH 7), the N-terminal propeptide of BinA folds into a alpha-helix that fits in a hydrophobic groove at the surface of BinB, in-between its PFD and C-terminal pro-peptide. In the pH 10 structure, the propeptide (L3-F10) is observed in an extended conformation, which could increase its accessibility to proteases.
I11-L157	**Trefoil**		Trefoil domain.
P158-F353	**PFD**		Pore-forming domain.
K241-E296	**TM sub-domain of the PFD**		In the complex structure, residues K241-E296 participate both in cementing the interaction between the trefoil and PFD domains of BinA, and in the dimerization with BinB.
A354-N370[18]	**C-terminal pro-peptide**		Residues 354-365 (out of 354-370) of BinA C-terminal propeptide are visible in the electron density.

Mutations (engineered or naturally observed)									
Mutation (with respect to strain A2)	Domain	Putative effect in structure	Effect on toxicity (*Culex* species)	Effect on complex formation	Effect on receptor binding	Effect on host range	A / B	Reference	Notes and Remark
C31S/A C47S/A	Trefoil cap (α carbohydrate-binding module)	Rupture of this disulphide bridge will likely result in destabilization of the α carbohydrate-binding module.	**Non toxic**	None	Presumably none *	n/a	n	[2]	That this mutation is active supports the hypothesis that the **α- module participates in the toxic action of BinA**.
S48N (strain 5)	Trefoil cap (α carbohydrate-binding module)	This residue is constitutive of the α carbohydrate-binding module.	Toxic	None	Presumably none	n/a	n	[5,19,20]	That this mutation is active supports the hypothesis that the **α- module participates in the toxic action of BinA**.
N52H/A	Trefoil cap (α carbohydrate-binding module)	This residue is constitutive of the α carbohydrate-binding module. Specifically, it supports the structuration of the adjacent trefoil sub-domain by hydrogen bonding its side chain nitrogen to the carbonyl oxygen of A34 (2.8 Å)	Slightly reduced toxicity	Presumably none	Minor	n/a	n	[1]	That this mutation is active supports the hypothesis that the **α- module participates in the toxic action of BinA**.
Q53	Trefoil cap (α carbohydrate-binding module)	This residue is constitutive of the α carbohydrate-binding module. Specifically, its side chain nitrogen and oxygen H-bond to the main chain oxygen and nitrogen of I46 and S47, respectively.	Slightly reduced toxicity	Presumably none	Minor	n/a	n	[1]	That this mutation is active supports the hypothesis that the **α- module participates in the toxic action of BinA**.
L93S (strain 4)	Trefoil cap (β carbohydrate-binding module)	This residue is constitutive of the β carbohydrate-binding module. Replacement by a serine would open a route to the hydrophobic core of the trefoil domain (F96, I110), possibly resulting in a collapse of the trefoil domain. It could also perturb the structure of the cap by destabilizing the F96-E114 beta-hairpin. Residues F96-A104 have been shown to encode the host range.	**Non toxic**	None	Presumably none	n/a	n	[3,5,19,20]	**L93S renders the toxin inactive** and may suppress BinA toxicity by three independent mechanisms. First, by inducing a collapse of the trefoil domain. Second, by disrupting the β carbohydrate-binding module. Third, by diminishing the host range of BinA, encoded around position 100.
R97A	Trefoil barrel (site IIIa carbohydrate-binding module)	R97 is at the beginning of a beta-hairpin (F96-E114) that contacts BinB. R97 does not, however, contribute to the interaction itself, nor does it directly participate to the folding of the trefoil. R97 is part of the site IIIa carbodyrate-binding module.	**Non toxic**	None	Presumably none	n/a	n	[4]	That the R97A mutation supresses toxicity supports the hypothesis that site IIIa is involved in the toxic function of BinA. **Site IIIa could be key to the host range.**
E98A	Trefoil barrel (inside surface)	E98 faces the interior of the barrel. Its OE1 and OE2 atoms are at 3.0 and 3.2 Å away from E14-OE1 and R19-NH1, respectively. E98-OE1 is also at 3.1 Å from Gln336-OE1. The mutation could thus effect either by reducing complexation with BinB or by destabilizing the barrel structure of BinA.	Reduced toxicity	None	Presumably none		y	[4]	

V99F (strain 3)	Trefoil barrel (site IIIa carbohydrate-binding module)	V99 is part of the site IIIa module. That its replacement by a Phe results in only slightly reduced toxicity highlights that a hydrophobic residue is required at this site to allow interaction wit the host.	Slightly reduced toxicity	None	Presumably none	Reduced host range	y	[3,5,19,20]	That the V99A mutation reduces toxicity supports the hypothesis that site IIIa is involved in the toxic function of BinA. **Site IIIa could be key to the host range.**
R101A	Trefoil barrel (outside surface)	R101 supports the architecture of the trefoil domain by stabilizing its last loop (T136-K150) through hydrogen-bonding interactions between its NH1 and NH2 atoms with carbonyl oxygens of N142 (3.1 Å distance) and I139 (2.8 Å distance), respectively. Furthermore, its main chain oxygen is involved in the BinAB dimer interface (Supplementary Table 4).	Slightly reduced toxicity	Reduced complexation	Presumably none	n/a	y	[4]	The mutation only slightly affects the toxicity of BinA, while the effect on the interaction with BinB is dramatic. This suggests that mutating R101 into an alanine results in a somewhat different trefoil structure that does not interact in the same fashion with BinB.
A104E/S (strain 1 / strains 3 & 4)	Trefoil barrel (outside surface)	A104 is at the tip of a beta-hairpin that establishes contact with BinB. Together with M103, it contribute to complexation with BinB; specifically, A104 establishes hydrophobic contact with BinB I446.	Reduced toxicity	These mutations are likely to weaken the interaction between BinA and BinB	Presumably none	Reduced host range	y	[3,5,19,20]	This mutation is found in strains 1 and 3, which are both less active than strain 2 and display smaller host range. It is also found in strain 4.
E114A	Trefoil barrel (outside surface)	E114 sits at the tip of beta hairpin (T105-E123) that is involved in the formation of both the site IIIa and γ carbohydrate-binding modules. Yet, the residue is neither part of the two modules, nor involved in complexation with BinB or crystal packing.	Reduced toxicity	Presumably none	Presumably none	Presumably none	n	[4]	It is unclear in which fashion E114 contributes to the toxicity of BinA. The proximity with the site IIIa and γ modules suggests E114 could be involved the toxic function of BinA.
P124T (strain 5)	Trefoil cap (γ carbohydrate-binding module)	P124 cycle is stacked above the aromatic cycle of F23 (closest distance between non-H atoms: 3.7 Å) and is constitutive of the γ carbohydrate-binding module. Mutation into a threonine is not expected to disrupt the folding of the trefoil.	Toxic	Presumably none	Presumably none	Presumably none	n	[5,19,20]	This strain was tested on *Culex* species only; hence, it remains to be determined whether the mutation could also affect the host range.
H125N (strains 3 and 4)	Trefoil cap (γ carbohydrate-binding module	H125 cycle is stacked over Y334 aromatic cycle (distance between two closest non-H atoms: 3.5 Å) and is constitutive of the γ carbohydrate-binding module. The mutation into an asparagine is not expected to impact the structure of the trefoil domain, nor the complexation with BinB, but could affect binding into the γ module. Of note, H125 is also involved in crystal packing interaction (Supplementary Table 8); the mutation could thus impact crystal formation.	Toxic	Presumably none	Presumably none	Reduced host range	n	[5,19,20]	This mutation is found in strain 3, which are both less active than strain 2 and displays smaller host ranges. It is also found in the inactive strain 4.
Y135F (strains 3 and 4)	Trefoil cap	Y135 is found at the pinnacle of the trefoil cap, at the junction between the α-, β-, and γ-carbohydrate binding modules. Specifically, Y135 stabilizes the cap of the trefoil domain by contributing an hydrogen bond between its hydroxyl oxygen and H33 main chain nitrogen. This H-bond directly associates the α- and γ-carbohydrate binding modules. The Y135F mutation would effect in suppressing this hydrogen bond. Hydrophobic interactions within the trefoil cap pinnacle would however remain unchanged.	Toxic	Presumably none	Presumably none	Presumably none	n	[5,19,20]	The effect on receptor binding, toxicity, host range, and complex formation is unknown, but presumably insignificant.
C195A	Loop-helix motif in the upper-part of the PFD sheet domain	C195 is at the second residue of a loop–helix motif that spans residues P194-Y213 and which contributes to the major interface between the trefoil and PFD domain. C195 sulphur sits in a hydrophobic pocket and supports the folding of into an alpha helix of	Reduced toxicity	Presumably none	Presumably none	Presumably none	n	[2]	By reinforcing the hydrophobicity at the interface between the trefoil and the PFD domains, the C195A mutation strengthen the stabilization of the trefoil domain over the PFD.

		segment I202-P212. Of note, the other interface is contributed by residues G269-K284, belonging to the TM of the PFD (K241-E296) and which participate both in associating the trefoil and PFD domains of BinA, and in the dimerization with BinB.							
M197R (strain 6)	Loop-helix motif in the upper-part of the PFD sheet domain	M197 is found at the hydrophobic interface between the between the trefoil and the PFD domain of BinA. Its replacement by an arginine is likely to provoke instability at the interface between the two domains. That this strain is more toxic than the others, which all bear a methionine at this position, suggest that disruption of the interaction between the trefoil and the PFD domain is an important step in carrying on the toxic activity.	**More toxic**	Presumably none	Presumably none	Presumably none	n	[5,19,20]	That this mutation result in increased toxicity suggests that a destabilization the interface between the PFD and the trefoil could be involved in the toxic action, e.g. by allowing a hinge motion of the trefoil over the PFD that could be required for pore formation by BinA. Interestingly, a methionine is found at the equivalent position in Cry35Ab1, a structurally related insecticidal toxin from *B. thuriengensis*. In BinB, which is not toxic, the equivalent residue is an isoleucine and the overall region is more hydrophobic. This observation supports the hypothesis that the relative hydrophobicity of this residue (and by extension, of the interface between the trefoil and the PFD) controls the toxicity of BinA.
R267K	TM subdomain of the PFD	This residue is found in the middle of the putative TM domain of the PFD (K241-E296) and participates in the dimerization with BinB. R267 features a hydrogen bond to BinA T290 main chain oxygen, highlighting the role of R267 in self-stabilizing the TM. The mutation into a Lys is not expected to affect the function of BinA, given that the latter H-bond would be preserved. It should also not affect the dimerization with BinB.	Toxic	Presumably none	Presumably none	Presumably none	y	[5,19,20]	
293-LLI-295 → AAA	TM subdomain of the PFD	This stretch of hydrophobic residues is found the C-terminal end of the TM subdomain of the PFD. I295 directly contacts I141 from BinB, thus weakly participating to complex formation. L294 faces the interior of the TM (K241-E296), which it stabilizes by hydrophobic interaction with buried residues I244, I260 and F264.	Reduced toxicity.	Presumably weak.	Presumably none	Presumably none	n	[1]	The mutations could affect the proper folding of BinA, which would explain the observed reduction of toxicity. By reducing the hydrophobicity of the TM, these mutations could also affect pore formation.
E302I/A	Sandwich subdomain of the PFD	This residue is found at the very end of the PFD domain, remote from regions involved in complexation with BinB.	**Non toxic**	Presumably none	Presumably none	Presumably none	n	[1]	The mutations are not expected to affect folding of BinA nor crystal packing and complexation with BinB. Thus, they probably abolish the toxic function by impeaching interaction with the host.
R312K/H/I/A	Sandwich subdomain of the PFD	R312 is found at the very end of the PFD domain, remote from the regions involved in the complexation with BinB. As compared to E302, R312 faces the other side of the PFD.	**Non toxic**	Presumably none	Presumably none	Presumably none	n	[1]	The mutations are not expected to affect folding of BinA nor crystal packing and complexation with BinB. Thus, they probably abolish the toxic function by impeaching interaction with the host.
325-HH-327 → AA	Sandwich subdomain of the PFD	The two residues are found in the PFD, just below the loop- helix motif P194-Y213. Likely, it is by destabilizing the latter subdomain that these mutations suppress the toxin toxicity.	**Non toxic**	Presumably none	Presumably none	Presumably none	n	[1]	

Supplementary Table 2 | BinB Structure-Function Summary Table

Domain and Subdomain boundaries			
M1-Y21[659]	**N-terminal pro-peptide**		None of the residues from BinB N-terminal propeptides are visible in electron density maps.
P35-I201	**Trefoil**		Trefoil domain.
P202-Y395	**PFD**		Pore-forming domain.
T289-E343	**TM sub-domain of the PFD**		In the complex structure, residues T289-E343 participate both in cementing the interaction between the trefoil and PFD domains of BinB, and in the dimerization with BinA.
P396-Q448[659]	**C-terminal pro-peptide**		The entire propeptide is visible in the electron density.

Mutations (engineered or naturally observed)									
Mutation (with respect to strain A2)	Domain	Putative effect in structure	Effect on toxicity (*Culex* species)	Effect on complex formation	Effect on receptor binding	Effect on host range	A / B	Reference	Notes and Remark
16-KK-17 → AA 16-KKK-18 → AAA	N-terminal propeptide (residues 1-21)	n/a	Toxic	Presumably none	Presumably none	Presumably none	n	[1]	In the complex structure of BinAB, residues 1-27 are not visible, implying that they do not participate in dimerization nor in crystal packing interactions. Residues 19-27 have been observed in the structure of activated BinB, where they display a strange L-shaped fold that is involved in crystal packing interactions. Thus, residues 1-27 are probably not involved in the toxic function.
32-YNL-34 → AAA	Trefoil	Y32 and L34 participate in consolidating the interaction surface between the trefoil domain and the PFD domain. The hydroxyl oxygen of Y32 H-bonds to I326 main chain nitrogen and L238 main chain oxygen, whereas L34 sits in a hydrophobic pocket delimited by L88, I201, Y260 and the carbons atoms of K39. N33 participates to the biological dimerization by contributing an hydrogen bond to T285 main chain oxygen.	Non-toxic	These mutations are likely to weaken the interaction between BinA and BinB	These mutations are likely to weaken binding of BinB to the receptor	Presumably none	y	[1,21]	The three residues are crucial. A destabilization of the contact interface between the trefoil and the PFD domain is likely to affect both the binding of BinB to the receptor, and the dimerization with BinA. Specifically, BinA N33 is central to the dimerization with BinB (Supplementary Table 4). It is thus not surprising that the triple mutant show no activity.
35-PEI-37 → AAA	Trefoil	P35 contributes a kink that corresponds to the transition between the N-terminal loop and the trefoil domain. Residues P35-K39 are indeed folded as an alpha helix, whereas K40 starts the first beta-strand of the trefoil domain.	Non-Toxic	Presumably none	None	Presumably none	y	[21–23]	While P35A, E36A and I37A each retain toxicity, the triple mutant is completely inactive. Since this mutant retains the ability to bind to the receptor, the likelihood is high that P35A, E36A and I37A are involved in another step required for the toxicity.
38-SKK-40 → AAA	Trefoil	These residues are located in the short alpha-helix motif that connects the N-terminal loop to the first strand of the trefoil domain. The mutations are likely to affect the proper folding of BinB trefoil. In addition, K39 and K40 are involved in crystal packing interactions (Supplementary Table 9).	Non-toxic	Presumably none	None	Presumably none	N	[1,21,22]	While completely inactive, this mutant retains the ability to bind to the receptor, suggesting that residues 38-40 are involved in another step required for the toxicity.
41-FYN-43 → AAA	Trefoil barrel (outside surface)	These residues are found on one of the strands constitutive of the barrel. Their side chains are located on the outside surface of the barrel.	Non-toxic	Presumably none	The mutation could effect by disrupting the receptor epitope on BinB.	Presumably none	n	[23]	While F41A, Y42A and I43A each retain toxicity, the triple mutant is completely inactive, possibly due to a complete disruption of the receptor epitope on BinB.
P35A	Trefoil	This residue is located in the short alpha-helix motif that connects the N-terminal loop to the first strand of the trefoil domain. P35 plays a mere supporting role in the dimerization with BinA, suggesting a minor effect on complex formation.	Slightly reduced toxicity	Presumably low.	Presumably none	Presumably none	y	[12]	
E36A	Trefoil	This residue is located in the short alpha-helix motif that connect the N-terminal loop to the first strand of the trefoil domain.	Slightly reduced toxicity	Presumably none	Presumably none	Presumably none	n	[12]	
I37A	Trefoil	This residue is located in the short alpha-helix motif that connect the N-terminal loop to the first strand of the trefoil domain.	Toxic	Presumably none	Presumably none	Presumably none	n	[12]	

F41A	Trefoil barrel (outside surface)	This residue is found on the outside surface of the trefoil barrel.	Reduced toxicity	Presumably none	The mutation could effect by disrupting the receptor epitope on BinB.	Presumably none	n	[12]	
Y42A	Trefoil barrel (outside surface)	This residue is found on the outside surface of the trefoil barrel.	Reduced toxicity	Presumably none	The mutation could effect by disrupting the receptor epitope on BinB.	Presumably none	n	[12]	
N43A	Trefoil barrel (outside surface)	This residue is found on the outside surface of the trefoil barrel.	Toxic	Presumably none	Presumably none	Presumably none	n	[12]	
52-GYG-54 → AAA	Trefoil barrel	The residues are located in a beta-turn of the trefoil domain. Since it was shown that the block mutation does not impact binding to the receptor, these residues are likely not involved in receptor binding.	Presumably toxic	Presumably none	Presumably none	Presumably none	n	[22]	
C67A/S C161A/S	Trefoil cap	C67 is located in the S56-M75 beta-hairpin, and C161 in the N160-T168 beta-turn. The C67-C161 disulphide bridge tethers in place a loop that prevents access to the α module.	Non-toxic	Presumably none	These mutations likely abolish the binding of BinB to the receptor.	Presumably none	n	[7]	
81-PRF-83 → AAA	Trefoil cap	These residues are C-terminal to the α carbohydrate-binding module. Since it was shown that this mutation does not impact the binding to the receptor, the trefoil sub-domain of which they are a part is probably not involved in receptor binding. Of note, these residues are involved in crystal packing interactions with another BinB molecule.	Presumably toxic	Presumably none	Presumably none	Presumably none	n	[8]	
85-IRF-87 → AAA	Trefoil barrel	These residues are found on one of the strands constitutive of the barrel. R86 faces the inside of trefoil barrel, whereas I85 and F87 contribute to its outside surface. Together with F41 and I95, the latter contribute a large hydrophobic surface that glues over PFD residues I242, T259, Y260 and M255. The block mutation likely affects both the structuration of the trefoil barrel (R86) and its cementing over the PFD.	Presumably not toxic	Presumably reduced (unfolded BinB likely presents reduced affinity for BinA)	These mutations abolish the binding of BinB to the receptor	Presumably none	n	[8]	The mutation could prevent the proper folding of BinB into a two-domain toxin, thus preventing dimerization with BinA. That binding to the receptor is abolished furthermore suggests that these residues could be part of the receptor epitope on BinB.
111-YLD-113 → AAA	Trefoil cap	The residues are located in a loop at the surface of the trefoil cap and are probably not involved in receptor binding.	Toxic	Presumably none	None	Presumably none	n	[21]	
115-NNH-117 → AAA	Trefoil cap	The residues are located in a loop at the surface of the trefoil cap and are probably not involved in receptor binding.	Toxic	Presumably none	None	Presumably none	n	[21]	
N114A	Trefoil cap	The residues are located in a loop at the surface of the trefoil cap and are probably not involved in receptor binding.	Toxic	Presumably none	None	Presumably none	n	[21]	
143-GEQ-145 → AAA	Trefoil barrel (outside surface)	These residues are located in a loop that contacts BinA. The mutation is thus expected to weaken the binding of BinB to BinA.	Reduced toxicity	Presumably reduced	Presumably reduced	Presumably none	n	[21]	The S137-F146 loop is folded back onto the trefoil domain in the structure of activated BinB without BinA (3WA1)
F146A	Trefoil barrel (outside surface)	F146 is exposed at the surface of the trefoil domain. Given that this mutation does not affect binding to BinA nor the toxicity, it is likely that F146 is not part of the receptor's binding site.	Toxic	Presumably none	None	Presumably none	n	[21]	
147-FQF-149 → AAA	Trefoil barrel (inside surface)	These residues are found on one of the strands constitutive of the trefoil barrel. Q148 faces the outside of barrel, whereas I85 and F87 contribute to its core. The block mutation renders the toxin unable to bind to the receptor. The loss of	Presumably not toxic	Presumably reduced (unfolded BinB likely presents	These mutations abolish the binding of BinB to the receptor	Presumably none	n	[8]	

		binding activity could be due an improper folding of the trefoil domain or to the disruption of the receptor epitope on BinB.		reduced affinity for BinA)					
147-FQFY-150 → AAAA	Trefoil barrel (inside surface)	These residues are found on one of the strands constitutive of the barrel. Q148 and Y150 face the outside of the barrel, whereas I85 and F87 contribute to its core. The block mutation renders the toxin unable to bind to the receptor. The loss of binding activity could be due an improper folding of the trefoil domain or to the disruption of the receptor epitope on BinB.	Not toxic	Presumably reduced	These mutations abolish the binding of BinB to the receptor	Presumably none	n	[21]	
F147A	Trefoil barrel (inside surface)	This mutant present reduced activity. The reduced binding activity could be due an improper folding of the trefoil domain or to the disruption of the receptor epitope on BinB.	Reduced toxicity	Presumably reduced	Slightly reduced binding of BinB to the receptor	Presumably none	n	[21]	
Q148A	Trefoil barrel (outside surface)	This mutant present reduced activity. The reduced binding activity could be due an improper folding of the trefoil domain or to the disruption of the receptor epitope on BinB.	Reduced toxicity	Presumably reduced	Slightly reduced binding of BinB to the receptor	Presumably none	n	[21]	
F149A	Trefoil barrel (inside surface)	The loss in binding activity could be due an improper folding of the trefoil domain or to the disruption of the receptor epitope on BinB.	Not toxic	Presumably reduced	Slightly reduced binding of BinB to the receptor	Presumably none	n	[21]	**IMPORTANCE OF AROMATIC RESIDUES AT THIS SPECIFIC LOCI**
F149Y	Trefoil barrel (inside surface)	That this mutation preserves activity infers that the aromaticity of F149 is crucial at this specific locus, to allow either a proper folding of the trefoil of BinB or recognition by the receptor. F149Y could further stabilize the barrel of BinB by contributing a H-bond to R86 side chain.	Slightly increased toxicity	Presumably none	Presumably increased binding	Presumably none	n	[21]	**IMPORTANCE OF AROMATIC RESIDUES AT THIS SPECIFIC LOCI**
Y150A	Trefoil barrel (outside surface)	The loss in binding activity could be due an improper folding of the trefoil domain or to the disruption of the receptor epitope on BinB.	Not toxic	Presumably reduced	Markedly reduced binding of BinB to the receptor	Presumably none	n	[21]	**IMPORTANCE OF AROMATIC RESIDUES AT THIS SPECIFIC LOCI**
Y150F	Trefoil barrel (outside surface)	That this mutation preserves activity infers that the aromaticity of Y150 is crucial at this specific locus, to allow either a proper folding of the trefoil of BinB or recognition by the receptor. Y150 is part of the site IIIa module of BinB suggesting that this site could play an important role in binding the receptor by hydrophobic interaction.	Slightly increased toxicity	Presumably none	Presumably increased binding	Presumably none	n	[21]	**IMPORTANCE OF AROMATIC RESIDUES AT THIS SPECIFIC LOCI**
207-TSL-209 → AAA	Trefoil	These residues are part of the long beta strand coming all the way from the trefoil domain down to the tip of the PFD (A190-L219). In the complex structure, L209 shows a weak interaction with F6 from BinA's N-terminal loop.	Presumably toxic	Presumably none	Presumably none	Presumably none	n	[8]	
231-RAV-233 → AAA	PFD	The residues are part of a beta strand that encompasses the sandwich and sheet sub-domains of the PFD domain. Anecdotally, these residues are spatially close to 207-TSL-209.	Presumably toxic	Presumably none	Presumably none	Presumably none	n	[8]	
C241A/S	Loop-helix motif in the upper-part of the PFD sheet domain	C241 is at the second residue of a loop–helix motif spanning residues P240-Y260 and which contributes to the major interface between the trefoil and PFD domain. C240 sulphur sits in a hydrophobic pocket and supports the folding of segment N251-T259 into an alpha helix, notably by contributing a sulphur-sulphur interaction with M255 side chain. Of note, the two other interfaces that associate the trefoil and PFD domains of BinB are the	Reduced toxicity	Presumably none	Presumably none	Presumably none	n	[7]	

		N-terminal loop and the TM domain (T289-E343). The residue equivalent to C241 in BinB is C195 in BinA. Both sit at the exact same place in the loop–helix motif. It is unclear what the exact role of this region is in BinB.							
L314H/Y (strain 1 / strain 3) F317L (strains 1 and 3)	TM subdomain of the PFD	These residues are found in the putative TM subdomain of BinB and are key to determining the host range. The BinAB toxins from strains 1 and 3 are active against *Culex pipiens, Culex quinquefasciatus* and *Aneopheles stephensi,* but not against *Mansonia uniformis, Anopheles aegypti, Aedes atropalpus.* The toxin from strain 2 is active against the whole range of hosts. The reverse-engineering of L314 (and F317) in BinAB toxins from strain 1 and strain 3 confers them with the host range of strain 2. In accordance with their likely structural role in toxicity, the two residues are exposed. The equivalent motif in BinA encompasses residues K241-E296.	Reduced toxicity	Presumably none	Presumably reduced	Reduced host range	n	[5]	
387-YRL-389 → AAA	Sandwich subdomain of the PFD	These residues are in a beta turn that associate the sandwich, sheet and propeptide domains of the PFD. The hydroxyl oxygen of Y387 interacts with D419 (sandwich/pro-peptide), while R388 side chain nitrogens H-bond to D310 main chain oxygen (sandwich/sheet) and to R367 and D386 side chains nitrogen and oxygen, respectively (sandwich/sandwich). In addition, L389 contribute to the hydrophobic groove in which, at the surface of BinB, the alpha helix formed by BinA residues F6-E14 sits. Thus, these residues are involved both in maintaining the cohesiveness of the PFD domain and in stabilizing the interaction with BinA. It was shown that these residues have no effect on receptor binding.	Non-toxic	Presumably lower	Presumably lower	Presumably none	y	[1]	
392-IQ-393 → AA	Sandwich subdomain of the PFD	These residues are part of an exposed beta strand of the PFD sandwich. I392 contribute hydrophobic interactions that stabilize the propeptide domain. Q393 is involved in stabilizing the long beta strand coming all the way from the trefoil domain down to the tip of the PFD (A190-L219). Surely, the effect of the Q393A mutation is stronger than that of the I292A.	Non-toxic	Presumably none	Presumably lower	Presumably none	n	[1]	
408-KH-409 → AA	C-terminal propeptide	This mutation affects the trypsin cleavage site of the BinB propeptide and probably lowers the propensity of BinA and BinB to undergo their presumably collective conformational change upon crystal dissolution. That the toxin remains active even in the presence of this large propeptide is in agreement with the hypothesis that the latter is only cleaved after – and not before – binding of BinB to the receptor.	Reduced toxicity	Presumably higher	Presumably lower	Presumably none	n	[1]	

Supplementary Table 3 | Contacts between BinA (x,y,z) and BinB (x,y,z) < 4Å

BinA (x,y,z)						BinB (x,y,z)									
chain	residue no.	residue name	atom in closest contact	subdomain location	buried area (Å²)	chain	residue no.	residue name	atom in closest contact	subdomain location	buried area (Å²)	sum area buried (Å²)	closest contact (Å)	interaction type	pH 10 sensitive?
A	3	ASN	ND2	PEP	27.4	B	212	ASN	OD1	SAN	29.4	56.8	3.5	POLR	N
A	4	LEU	CD2	PEP	23.6	B	210	LEU	CD2	SHE	22.2	45.8	3.8	HPHO	N
A	4	LEU	CD2	PEP	14.4	B	211	GLU	O	SHE	4.6	19.0	3.5	POLR	N
A	4	LEU	N	PEP	25.0	B	212	ASN	ND2	SAN	33.9	58.9	3.5	POLR	N
A	4	LEU	CD2	PEP	14.7	B	269	TRP	CZ2	SHE	6.4	21.1	3.4	HPHO	N
A	4	LEU	CD1	PEP	13.0	B	434	VAL	CG2	PEP	13.2	26.2	3.8	HPHO	N
A	7	ILE	CG2	PEP	15.7	B	210	LEU	CD2	SHE	16.9	32.6	3.8	HPHO	N
A	7	ILE	CD1	PEP	13.5	B	269	TRP	CH2	SHE	5.2	18.7	3.8	HPHO	N
A	7	ILE	CD1	PEP	15.6	B	434	VAL	CG1	PEP	12.5	28.1	3.9	HPHO	N
A	8	ASP	OD1	PEP	28.4	B	234	LYS	NZ	SHE	24.0	52.4	2.9	HBND	N
A	8	ASP	OD1	PEP	20.0	B	267	GLU	OE2	SHE	7.7	27.7	3.6	POLR	N
A	11	ILE	CG2	TRF	34.5	B	388	ARG	NH2	SAN	26.2	60.7	4.0	POLR	N
A	11	ILE	CG1	TRF	22.7	B	389	LEU	CD1	SAN	13.2	35.8	3.9	HPHO	N
A	11	ILE	CD1	TRF	17.2	B	436	VAL	CG1	PEP	11.7	28.8	3.8	HPHO	N
A	11	ILE	CD1	TRF	21.5	B	438	THR	OG1	PEP	15.8	37.3	3.9	POLR	N
A	14	GLU	CG	TRF	18.2	B	336	GLN	OE1	SHE	21.9	40.1	3.4	POLR	N
A	22	ASP	OD2	TRF	12.8	B	448	GLN	O	PEP	22.8	35.6	2.9	HBND	Y
A	61	ASP	O	TRF	25.3	B	324	GLU	CG	TM	26.6	52.0	3.2	POLR	N
A	61	ASP	OD1	TRF	15.5	B	328	ARG	NH2	TM	21.2	36.7	3.5	POLR	N
A	62	ASP	CA	TRF	15.0	B	324	GLU	OE2	TM	17.1	32.2	3.8	POLR	N
A	98	GLU	O	TRF	28.5	B	336	GLN	NE2	SHE	35.7	64.2	3.6	POLR	N
A	99	VAL	CA	TRF	8.9	B	336	GLN	O	SHE	22.9	31.8	3.4	POLR	N
A	100	LYS	N	TRF	28.5	B	336	GLN	O	SHE	47.6	76.1	2.8	HBND	N
A	100	LYS	O	TRF	19.4	B	337	THR	OG1	SHE	10.1	29.5	3.0	HBND	N
A	100	LYS	CB	TRF	19.6	B	418	TYR	OH	PEP	23.7	43.3	3.6	POLR	N
A	101	ARG	O	TRF	13.0	B	418	TYR	CD1	PEP	15.8	28.7	3.4	POLR	N
A	102	THR	CG2	TRF	9.7	B	417	SER	O	PEP	12.3	21.9	3.9	POLR	N
A	102	THR	CA	TRF	26.3	B	418	TYR	O	PEP	26.4	52.6	3.5	POLR	N
A	103	MET	N	TRF	37.5	B	418	TYR	O	PEP	26.9	64.4	2.9	HBND	N
A	103	MET	SD	TRF	18.5	B	446	ILE	CD1	PEP	19.4	37.9	3.7	POLR	N

A	103	MET	CE	TRF	24.5	B	419	ASP	OD2	PEP	14.8	39.3	3.2	POLR	N
A	103	MET	CE	TRF	12.0	B	387	TYR	OH	SAN	3.5	15.5	3.8	POLR	N
A	104	ALA	CB	TRF	22.9	B	446	ILE	CG1	PEP	19.2	42.1	3.7	HPHO	N
A	104	ALA	CB	TRF	13.3	B	421	HIS	NE2	PEP	8.7	22.0	4.0	POLR	N
A	148	SER	OG	TRF	16.0	B	448	GLN	O	PEP	21.9	37.9	3.2	POLR	N
A	149	ASN	O	TRF	15.7	B	447	ASN	ND2	PEP	22.4	38.1	2.8	HBND	N
A	150	LYS	O	TRF	17.4	B	447	ASN	O	PEP	24.2	41.6	3.4	POLR	N
A	150	LYS	CG	TRF	15.7	B	448	GLN	OXT	PEP	24.9	40.6	3.5	POLR	N
A	152	GLN	OE1	TRF	20.3	B	447	ASN	ND2	PEP	22.3	42.6	3.2	HBND	N
A	262	ALA	CB	TM	30.1	B	140	TYR	CD1	TRF	24.0	54.1	3.6	HPHO	N
A	263	ASP	CB	TM	19.3	B	140	TYR	CE2	TRF	21.6	40.8	3.9	HPHO	N
A	277	GLY	C	TM	23.1	B	328	ARG	NH2	TM	24.9	48.0	3.5	POLR	N
A	278	GLY	N	TM	11.3	B	328	ARG	NH2	TM	19.0	30.3	3.7	POLR	N
A	280	THR	O	TM	5.7	B	33	ASN	ND2	TRF	14.6	20.3	3.1	HBND	N
A	281	TYR	CE2	TM	14.1	B	31	ASN	O	TRF	22.5	36.6	3.6	POLR	N
A	281	TYR	CE2	TM	21.3	B	32	TYR	O	TRF	13.9	35.2	3.7	POLR	N
A	281	TYR	OH	TM	31.3	B	328	ARG	O	TM	36.3	67.6	3.5	POLR	W
A	281	TYR	OH	TM	17.3	B	329	GLY	CA	TM	12.7	30.0	3.5	POLR	N
A	284	LYS	NZ	TM	33.5	B	31	ASN	O	TRF	34.4	67.9	2.8	HBND	N
A	284	LYS	C	TM	25.6	B	33	ASN	OD1	TRF	26.2	51.8	3.7	POLR	N
A	285	THR	O	TM	3.8	B	33	ASN	ND2	TRF	14.5	18.3	2.8	HBND	N
A	286	GLN	NE2	TM	30.6	B	33	ASN	ND2	TRF	17.2	47.9	3.1	HBND	N
A	286	GLN	OE1	TM	27.1	B	35	PRO	CG	TRF	26.2	53.3	3.4	POLR	N
A	289	ASN	ND2	SHE	34.4	B	134	GLN	O	TRF	30.5	64.9	2.8	HBND	N
A	289	ASN	O	SHE	22.5	B	135	VAL	CA	TRF	6.9	29.4	3.4	POLR	N
A	289	ASN	O	SHE	24.2	B	136	GLY	N	TRF	14.9	39.1	2.8	HBND	N
A	290	THR	OG1	SHE	12.3	B	136	GLY	O	TRF	15.9	28.2	3.0	HBND	N
A	290	THR	CB	SHE	12.2	B	140	TYR	OH	TRF	19.4	31.6	3.6	POLR	N
A	292	GLN	NE2	SHE	21.9	B	138	GLY	O	TRF	24.6	46.5	2.8	HBND	N
A	297	THR	OG1	SAN	14.9	B	141	ILE	CG2	TRF	24.3	39.2	3.4	POLR	N
A	299	TYR	OH	SAN	26.1	B	141	ILE	O	TRF	28.7	54.8	2.7	HBND	Y
A	364	ILE	CD1	PEP	25.2	B	141	ILE	CD1	TRF	22.8	48.0	3.8	HPHO	N
A	364	ILE	CD1	PEP	15.8	B	142	THR	CG2	TRF	12.0	27.8	3.8	HPHO	N

Supplementary Table 4 | Contacts between BinA (x,y,z) and BinA (x+1/2,-y+1/2,-z) < 4Å

BinA (x,y,z)						BinA (x+1/2,-y+1/2,-z)									
chain	residue no.	residue name	atom in closest contact	subdomain location	area buried (Å²)	chain	residue no.	residue name	atom in closest contact	subdomain location	area buried (Å²)	sum area buried (Å²)	closest contact (Å)	interaction type	pH 10 sensitive?
A	24	TYR	OH	TRF	16.2	A	329	GLY	O	SHE	17.4	33.6	3.8	POLR	W
A	27	GLU	OE1	TRF	23.1	A	184	GLU	CB	SHE	25.9	49	4	POLR	N
A	27	GLU	OE2	TRF	31.1	A	164	GLU	CD	SHE	34.2	65.2	3.3	POLR	N
A	28	TYR	CE1	TRF	37.4	A	184	GLU	OE2	SHE	36.7	74.1	3.4	POLR	N
A	102	THR	CG2	TRF	18.7	A	328	ASP	OD2	SHE	13.6	32.3	3.8	POLR	N
A	102	THR	CG2	TRF	20.3	A	330	THR	CG2	SHE	16.7	37	3.4	HPHO	N
A	141	ASN	O	TRF	21.4	A	158	PRO	CA	SHE	8.6	29.9	3.4	POLR	N
A	141	ASN	O	TRF	19.4	A	159	SER	N	SHE	15.5	35	3.3	POLR	N
A	141	ASN	OD1	TRF	25.7	A	327	SER	O	SHE	29.4	55.1	3.4	POLR	N
A	141	ASN	OD1	TRF	15.5	A	328	ASP	N	SHE	15.3	30.8	3.9	POLR	N
A	141	ASN	ND2	TRF	16.1	A	156	THR	O	TRF	22.6	38.8	3.6	POLR	N
A	142	ASN	OD1	TRF	8.9	A	159	SER	OG	SHE	23.1	32	3.2	POLR	N
A	143	SER	N	TRF	19.5	A	159	SER	OG	SHE	21.6	41.1	3.1	HBND	N
A	144	ASN	ND2	TRF	13.3	A	159	SER	OG	SHE	12.3	25.5	3.9	POLR	N

Supplementary Table 5 | Contacts between BinA (x,y,z) and BinB (x-1/2,-y+1/2,-z) < 4Å

BinA (x,y,z)						BinB (x-1/2,-y+1/2,-z)									
chain	residue no.	residue name	atom in closest contact	subdomain location	area buried (Å²)	chain	residue no.	residue name	atom in closest contact	subdomain location	area buried (Å²)	sum area buried (Å²)	closest contact (Å)	interaction type	pH 10 sensitive?
A	201	PHE	CB	SHE	32.8	B	411	ILE	O	PEP	34.2	67	3.4	POLR	N
A	201	PHE	CB	SHE	17.8	B	412	ILE	O	PEP	16.9	34.7	3.3	POLR	N
A	201	PHE	O	SHE	17.8	B	413	ARG	CA	PEP	7.4	25.3	3.7	POLR	N
A	201	PHE	CD1	SHE	30.8	B	414	CYS	O	PEP	17.7	48.5	3.4	POLR	N
A	201	PHE	CE1	SHE	12.7	B	416	ASN	O	PEP	6.2	18.9	3.7	POLR	N
A	202	ILE	CG1	SHE	7.2	B	414	CYS	O	PEP	12.7	19.9	3.4	POLR	N
A	203	SER	OG	SHE	18.3	B	415	GLU	OE1	PEP	27.9	46.2	3	HBND	N
A	206	GLU	CG	SHE	32.4	B	415	GLU	O	PEP	31.2	63.6	3.3	POLR	N
A	326	ARG	NH1	SHE	15	B	417	SER	CB	PEP	23	37.9	3.7	POLR	N
A	328	ASP	OD2	SHE	24.5	B	417	SER	OG	PEP	19.5	44	2.6	HBND	N
A	330	THR	OG1	SHE	12.7	B	417	SER	CB	PEP	20.1	32.9	3.9	POLR	N
A	331	GLN	OE1	SHE	38	B	444	PRO	CB	PEP	39.5	77.6	4	POLR	N
A	334	THR	O	SHE	35.8	B	443	ILE	CG2	PEP	33.8	69.6	3.9	POLR	N
A	334	THR	OG1	SHE	29.5	B	422	ILE	CD1	PEP	24.6	54.1	3.9	POLR	N

Supplementary Table 6 | Contacts between BinA (x,y,z) and BinB (-x+2, y-1/2,-z+1/2) < 4Å

BinA (x,y,z)						BinB (-x+2, y-1/2,-z+1/2)									
chain	residue no.	residue name	atom in closest contact	subdomain location	area buried (Å²)	chain	residue no.	residue name	atom in closest contact	subdomain location	area buried (Å²)	sum area buried (Å²)	closest contact (Å)	interaction type	pH 10 sensitive?
A	233	GLY	O	SAN	23.7	B	294	VAL	CG1	SHE	19	42.7	3.9	POLR	N
A	306	THR	CB	SAN	19.9	B	292	SER	OG	SHE	20.9	40.8	3.6	POLR	N
A	306	THR	CG2	SAN	16.9	B	294	VAL	CG2	SHE	23.7	40.6	3.9	HPHO	N
A	307	GLU	OE2	SAN	16	B	288	ARG	NH2	SAN	19.2	35.2	3.1	HBND	N
A	307	GLU	OE1	SAN	10.6	B	343	GLU	OE2	SAN	9.2	19.9	3.5	POLR	N
A	307	GLU	OE1	SAN	12.3	B	413	ARG	NH2	PEP	18	30.3	2.9	HBND	N
A	308	ASN	ND2	SAN	28.4	B	293	GLU	OE2	SHE	25.8	54.2	3.6	POLR	N
A	309	PHE	CE2	SAN	8.7	B	413	ARG	NH2	PEP	12.2	20.9	4	POLR	N
A	343	ASN	O	SAN	27.9	B	227	LYS	NZ	SHE	27.7	55.6	3.6	POLR	N
A	353	PHE	O	SAN	22.1	B	286	GLN	OE1	SAN	27.5	49.6	3.7	POLR	N
A	353	PHE	O	SAN	18.5	B	288	ARG	NH1	SAN	12.8	31.3	3.3	POLR	N
A	355	SER	CB	PEP	24.6	B	288	ARG	NE	SAN	23.4	48	3.4	POLR	N
A	355	SER	OG	PEP	5.1	B	343	GLU	OE1	SAN	4.2	9.3	5	POLR	N
A	358	LEU	CD2	PEP	16.6	B	295	VAL	CG2	SHE	18.6	35.3	3.8	HPHO	N

Supplementary Table 7 | Contacts between BinA (x,y,z) and BinB (-x+5/2, -y,-z+1/2) < 4Å

BinA (x,y,z)						BinB (-x+5/2, -y,-z+1/2)									
chain	residue no.	residue name	atom in closest contact	subdomain location	area buried (Å²)	chain	residue no.	residue name	atom in closest contact	subdomain location	area buried (Å²)	sum area buried (Å²)	closest contact (Å)	interaction type	pH 10 sensitive?
A	125	HIS	ND1	TRF	13.7	B	59	GLU	OE2	TRF	26	39.7	2.8	HBND	N
A	125	HIS	C	TRF	14.8	B	60	PHE	CZ	TRF	21.1	35.9	4	HPHO	N
A	126	SER	CB	TRF	12	B	60	PHE	CZ	TRF	16	28	3.6	HPHO	N
A	130	PRO	O	TRF	15	B	60	PHE	CE1	TRF	14.5	29.5	3.5	POLR	N
A	131	SER	CB	TRF	6.2	B	60	PHE	CE1	TRF	18.8	25.1	4	HPHO	N
A	132	ARG	NH1	TRF	18.7	B	59	GLU	CB	TRF	18.6	37.3	4	POLR	N
A	134	TYR	OH	TRF	23.4	B	59	GLU	OE2	TRF	26.2	49.6	3	HBND	Y

Supplementary Table 8 | Contacts between BinB (x,y,z) and BinB (-x+2, y-1/2,-z+1/2) < 4Å

BinB (x,y,z)						BinB (-x+2, y-1/2,-z+1/2)									
chain	residue no.	residue name	atom in closest contact	subdomain location	area buried (Å²)	chain	residue no.	residue name	atom in closest contact	subdomain location	area buried (Å²)	sum area buried (Å²)	closest contact (Å)	interaction type	pH 10 sensitive?
B	39	LYS	NZ	TRF	34.7	B	217	THR	OG1	SAN	36.2	70.9	2.9	HBND	N
B	39	LYS	NZ	TRF	17	B	218	SER	OG	SAN	13.4	30.4	3.3	POLR	N
B	40	LYS	CG	TRF	13.3	B	217	THR	CG2	SAN	20.2	33.5	3.7	HPHO	N
B	51	ASN	O	TRF	37.5	B	206	GLN	NE2	SHE	47.7	85.2	2.8	HBND	N
B	78	ASN	ND2	TRF	13.5	B	206	GLN	NE2	SHE	21.2	34.7	3.2	HBND	N
B	194	SER	C	TRF	20.8	B	230	VAL	CG2	SHE	21.2	42	3.9	HPHO	N
B	195	SER	OG	TRF	26.7	B	211	GLU	OE1	SHE	19.7	46.4	2.6	HBND	N
B	195	SER	OG	TRF	24	B	230	VAL	CG1	SHE	21.2	45.2	3.6	POLR	N
B	196	PHE	CE1	TRF	23.9	B	215	GLU	O	SAN	26	50	3.4	POLR	N
B	196	PHE	CE2	TRF	12.8	B	224	VAL	CG2	SAN	17.7	30.5	3.9	HPHO	N
B	197	TYR	CA	TRF	24.8	B	215	GLU	OE2	SAN	22.1	47	3.6	POLR	N
B	197	TYR	OH	TRF	27.9	B	212	ASN	OD1	SHE	36	63.9	2.6	HBND	N
B	198	ALA	N	TRF	26.7	B	215	GLU	OE2	SAN	28.5	55.2	2.9	HBND	N
B	198	ALA	CB	TRF	10.7	B	216	PRO	O	SAN	6.4	17.1	3.8	POLR	N
B	198	ALA	CB	TRF	14.8	B	217	THR	CG2	SAN	15.4	30.2	3.8	HPHO	N
B	201	ILE	CD1	SHE	16.7	B	217	THR	O	SAN	17.1	33.8	3.8	POLR	N
B	201	ILE	O	SHE	23	B	395	TYR	OH	SAN	24.5	47.6	2.9	HBND	Y
B	202	PRO	C	SHE	2.2	B	395	TYR	OH	SAN	14.5	16.7	3.9	POLR	N
B	203	GLN	CG	SHE	26.5	B	395	TYR	OH	SAN	24.2	50.7	3.5	POLR	N
B	203	GLN	OE1	SHE	21.7	B	356	LEU	CD2	SAN	21.2	42.9	3.5	POLR	N
B	203	GLN	OE1	SHE	21.8	B	396	PRO	CG	PEP	17.1	38.9	3.7	POLR	N
B	374	VAL	CG1	SHE	26.2	B	400	ILE	CD1	PEP	27.6	53.8	3.8	HPHO	N

Supplementary Table 9 | Contacts between BinB (x,y,z) and BinB (x, y-1, z) < 4Å

BinB (x,y,z)						BinB (-x+2, y-1/2,-z+1/2)									
chain	residue no.	residue name	atom in closest contact	subdomain location	area buried (Å²)	chain	residue no.	residue name	atom in closest contact	subdomain location	area buried (Å²)	sum area buried (Å²)	closest contact (Å)	interaction type	pH 10 sensitive?
B	50	ARG	NH2	TRF	37.3	B	355	ASP	CB	SAN	28.5	65.7	3.6	POLR	N
B	50	ARG	NH1	TRF	40.2	B	356	LEU	CD2	SAN	41.5	81.7	3.4	POLR	N
B	184	SER	CB	TRF	31.6	B	280	HIS	ND1	SAN	24	55.6	3.6	POLR	N
B	187	GLN	OE1	TRF	19.7	B	280	HIS	NE2	SAN	20.9	40.7	3.1	HBND	N
B	187	GLN	NE2	TRF	22.2	B	355	ASP	OD1	SAN	23.7	45.9	3.3	POLR	N
B	50	ARG	NH2	TRF	37.3	B	355	ASP	CB	SAN	28.5	65.7	3.6	POLR	N
B	50	ARG	NH1	TRF	40.2	B	356	LEU	CD2	SAN	41.5	81.7	3.4	POLR	N
B	184	SER	CB	TRF	31.6	B	280	HIS	ND1	SAN	24	55.6	3.6	POLR	N

Supplementary Table 10 | Terminal propeptide segments and their contributions to the heterodimer interface.

Region	Propeptide Residue Range (count)	Range observed in map	Heterdimer surface area lost by removing propeptide ($Å^2$)
BinA-Nterm	1-10 (10)	1-10 (10)	637
BinA-Cterm	354-370 (17)	354-365 (12)	56
BinB-Nterm	1-21 (21)	none (0)	0
BinB-Cterm	396-448 (53)	396-448 (53)	948
total	(101)	(75)	1539

Supplementary Table 11 | Negative and positive $F_o^{[pH10]}$ - $F_o^{[pH7]}$ peaks observed on BinA. Peaks stronger than ± 3.5 σ were integrated contiguously in the $F_o^{[pH10]}$ - $F_o^{[pH7]}$ map and then assigned to the closest residue (within a 1.5 Å radius) in the *pH7* (negative peaks) or *pH10* structures (positive peaks).

Negative peaks				
Residue name	Residue number	Atom name	Peak height (σ)	Integrated peak value (σ)
ASP	342	CG	-6.08	-309.84
GLY	15	O	-6.79	-251.42
THR	13	O	-4.73	-165.61
ARG	97	CG	-4.82	-132.95
SER	9	OG	-4.48	-88.96
HIS	127	CD2	-5.04	-83.22
GLU	98	OE2	-5.08	-65.03
ASN	69	N	-4.49	-62.95
GLU	45	CA	-4.15	-59.29
ILE	11	N	-4.35	-58.39
SER	126	OG	-4.25	-53.67
ASN	343	CB	-4.46	-52.56
LYS	16	CD	-4.48	-50.86
TYR	344	CE1	-4.06	-48.45
ASP	22	CG	-4.28	-39.32
LEU	129	CB	-4.29	-39.00
TYR	17	CA	-4.12	-35.19
GLU	98	CA	-4.24	-34.65
ALA	109	C	-4.11	-29.89
HIS	125	NE2	-4.11	-26.10
ARG	120	NH2	-3.97	-22.28
MET	103	CG	-3.74	-21.52
THR	330	CA	-4.09	-19.26
THR	102	CA	-4.12	-19.21
ASN	52	CB	-4.00	-18.73
TYR	107	CA	-3.95	-15.16
VAL	119	N	-3.89	-14.87
TYR	213	C	-3.79	-14.62
LEU	108	CA	-3.74	-14.56
MET	208	C	-3.86	-14.55
SER	320	C	-3.63	-14.35
PHE	95	CB	-3.66	-10.95
THR	210	CA	-3.77	-10.94
GLU	151	N	-3.85	-7.53
ASN	341	OD1	-3.90	-7.47

Positive peaks				
Residue name	Residue number	Atom name	Peak height (σ)	Integrated peak value (σ)
CYS	31	CB	5.53	149.34
ASP	22	CE	6.11	123.42
ASN	112	CB	4.29	118.04
LYS	16	CA	5.45	116.59
GLY	15	O	5.09	102.55
ASP	342	CA	4.97	93.51
LYS	209	CA	4.44	66.52
ASN	142	O	4.59	44.18
ASN	341	CD1	4.22	42.59
ILE	139	OE1	4.07	41.51
THR	13	CE2	4.30	38.27
ASP	8	CA	4.14	35.31
PRO	12	CD2	4.18	30.90
LEU	157	CB	4.07	26.96
GLU	98	CB	4.16	26.89
TYR	215	CB	4.04	23.05
ASN	343	CB	4.06	22.49
PHE	30	O	3.97	22.12
ARG	64	N	3.94	21.96
GLN	111	C	4.03	18.98
PRO	29	C	3.95	18.64
HIS	127	C	3.97	18.60
PHE	279	CG2	3.98	15.03
SER	126	C	3.81	14.67
ASP	118	CG	3.72	14.57
PRO	130	CG	3.87	11.05
LEU	340	ND1	3.73	10.85
ILE	110	O	3.66	10.74
SER	248	CG	3.59	10.69
GLN	286	CZ	3.72	7.26
PHE	96	CB	3.68	7.20
HIS	71	CE	3.63	7.16
ALA	121	CB	3.54	7.08
GLN	249	CA	3.56	7.06
PHE	264	O	3.51	7.01

ILE	66	CA	-3.70	7.28
GLY	205	C	-3.76	7.26
ILE	7	O	-3.70	-7.20
ARG	301	CB	-3.51	-7.02

Supplementary Table 12 | Negative and positive $F_o^{[pH10]}$ - $F_o^{[pH7]}$ peaks observed on BinB. Peaks stronger than ± 3.5 σ were integrated contiguously in the $F_o^{[pH10]}$ - $F_o^{[pH7]}$ map and then assigned to the closest residue (within a 1.5 Å radius) in the *pH7* (negative peaks) or *pH10* structures (positive peaks).

Negative peaks				
Residue name	Residue number	Atom name	Peak height (σ)	Integrated peak value (σ)
ASN	160	CA	-5.66	-147.51
GLY	321	N	-4.89	-130.14
LYS	47	CA	-4.82	-82.25
THR	151	OG1	-4.96	-81.95
MET	75	SD	-4.82	-75.13
ARG	129	O	-4.56	-63.77
PRO	182	CA	-4.62	-60.44
LEU	303	CA	-4.78	-59.56
ASN	78	N	-4.57	-55.80
PHE	87	C	-4.62	-54.69
ASP	419	OD2	-4.30	-49.29
PHE	41	CG	-4.51	-47.69
CYS	67	CB	-4.41	-45.80
CYS	241	C	-4.40	-44.19
GLU	103	O	-3.99	-41.36
THR	189	CG2	-4.39	-38.36
ASP	113	CA	-3.99	-37.18
LEU	334	O	-4.10	-34.25
ARG	231	C	-4.03	-33.91
ILE	435	CA	-4.15	-33.78
LYS	40	C	-3.94	-33.67
ILE	95	C	-4.08	-33.66
ILE	96	C	-4.35	-32.89
ALA	390	C	-4.07	-30.98
THR	410	CB	-4.12	-30.30
GLU	107	N	-4.02	-29.63
ASN	33	O	-3.81	-29.35
TYR	180	N	-3.97	-29.34
SER	49	N	-4.25	-26.84
GLN	448	C	-4.11	-26.39
THR	58	CG2	-4.06	-25.97
ALA	229	CB	-3.87	-25.81
ASP	165	CA	-3.78	-25.66
ILE	85	C	-3.92	-22.29
ASP	106	CG	-3.75	-21.87

Positive peaks				
Residue name	Residue number	Atom name	Peak height (σ)	Integrated peak value (σ)
HIS	117	NE2	5.31	232.86
ARG	167	O	5.64	196.87
GLN	187	OE1	5.45	173.12
CYS	161	CB	5.82	164.11
PRO	61	N	4.73	131.12
ARG	122	CB	4.76	124.12
HIS	245	CB	4.11	88.23
LEU	164	O	4.46	68.67
MET	255	SD	4.87	56.83
ASP	100	N	4.29	54.02
ILE	176	CB	4.37	50.50
GLU	59	O	4.19	50.37
GLU	347	OE2	4.47	46.98
ARG	167	NE	4.31	46.22
ASP	246	OD1	4.16	45.24
ASP	77	CA	4.13	42.13
ASN	116	N	4.29	41.87
GLU	368	OE1	4.28	38.41
SER	63	OG	4.17	38.03
ILE	64	CB	4.08	38.02
PRO	178	O	3.92	30.20
ILE	85	CD1	4.26	30.09
THR	186	O	3.98	30.02
PHE	87	CE2	4.05	26.83
GLN	296	N	4.07	26.42
ASP	106	OD1	4.01	22.44
ARG	129	CD	3.94	22.39
PHE	147	CD2	3.91	18.87
ARG	318	C	3.85	18.35
TYR	124	CZ	3.78	18.18
LYS	175	C	3.90	15.00
SER	320	N	4.02	14.99
ASN	381	OD1	3.89	14.92
VAL	230	CG1	3.90	14.89
ARG	288	NH2	3.82	14.60

GLU	133	C	-3.86	-18.81
GLN	286	CG	-3.90	-18.72
PRO	61	C	-4.04	-18.69
PRO	205	CG	-3.94	-18.66
ILE	120	CG1	-4.03	-18.43
ALA	97	C	-3.71	-18.06
ASP	220	OD1	-3.90	-15.32
TYR	76	C	-3.97	-15.32
SER	73	CA	-3.93	-15.01
THR	125	OG1	-3.91	-14.86
THR	173	CB	-3.76	-14.74
PRO	118	CG	-3.74	-14.63
ARG	167	C	-3.89	-14.57
ALA	163	CA	-3.84	-14.55
ASN	297	CG	-3.73	-14.52
ASN	78	CG	-3.73	-14.51
GLY	342	O	-3.70	-14.44
TYR	260	O	-3.66	-14.30
PHE	146	CD1	-3.85	-11.17
PRO	61	CA	-3.77	-11.06
LEU	158	C	-3.79	-10.89
GLN	130	CA	-3.66	-10.84
GLN	393	CB	-3.61	-10.71
ASN	331	N	-3.63	-10.69
THR	168	O	-4.14	-7.97
GLU	128	OE1	-3.80	-7.55
GLY	92	C	-3.76	-7.43
TYR	418	CD1	-3.81	-7.40
ILE	176	CA	-3.71	-7.37
ASN	160	CB	-3.71	-7.37
LYS	57	NZ	-3.74	-7.29
HIS	409	CG	-3.66	-7.28
TYR	48	CG	-3.71	-7.24
ASP	100	OD2	-3.61	-7.15
PHE	87	CZ	-3.57	-7.11
ARG	332	CB	-3.56	-7.11

TYR	53	CE1	3.73	14.58
GLN	325	CA	3.76	14.53
HIS	117	ND1	3.67	14.40
PRO	226	C	3.81	11.21
THR	327	OG1	3.77	11.12
PRO	396	CA	3.82	11.07
ARG	318	CG	3.80	11.06
LYS	284	O	3.76	11.01
ARG	99	N	3.69	10.95
ASN	160	ND2	3.76	10.89
ILE	169	CD1	3.65	10.75
LEU	389	CG	3.60	10.64
PHE	83	CG	3.84	7.48
LEU	171	N	3.83	7.40
TYR	418	CD2	3.80	7.39
ASN	352	OD1	3.79	7.32
ASN	51	CA	3.63	7.26
PHE	191	CD1	3.67	7.25
TYR	72	CD2	3.66	7.23
GLY	92	CA	3.60	7.13
ILE	169	CG1	3.56	7.11
GLU	103	CA	3.60	7.11
VAL	436	C	3.55	7.05

Supplementary Table 13 | Toxicity of wild type (BinAB) and mutated (BinA^{D22N}B) strains of *Lysinibacillus sphaericus* against 4th-instar larvae of *Culex quinquefasciatus* [a]. At the LC_{50} and LC_{95} levels, the mutant strain BinA^{D22N}B is 11.6 and 24 fold less toxic than the wild-type, respectively. An aspartic acid at position 22 in BinA is thus critical to the toxicity.

Bacterial Strain (Toxins produced)	LC_{50} (Fiducial Limits)	LC_{95} (Fiducial Limits)	Slope
***Lysinibacillus sphaericus* 4Q7/pBUSP-1 (BinAB)**	9.9 (7.1 – 13.4)	51.8 (32.9 – 116.1)	2.3 ± 0.4
***Lysinibacillus sphaericus* 4Q7/pBUSP-1 D22N (BinA^{D22N}B)**	115.5 (49.4 – 273.4)	1,268.2 (200.9 – 10,071.7)	1.6 ± 0.4

[a] 48 hr-mortality, ng/ml

Supplementary Table 14 | Refinement statistics for the model obtained from Molecular Replacement.

	Native, pH 7
Refinement	
Refinement target function	Maximum-likelihood
Resolution (Å)	2.25 (2.31-2.25)
Number of reflections	52257
R_{work} / R_{free}	0.158 / 0.203
Number of atoms	
Protein	6340
Water	283
B-factors (Å^2)	
Protein (BinA/BinB)	47.1 / 41.1
Water	43.8
R.m.s. deviations	
Bond lengths (Å)	0.01
Bond angles (°)	1.2