# Toxicity Mechanisms of Two Disparate Pore-Forming Toxins (Friend & Foe) from *Bacillus thuringiensis* and *Bordetella pertussis*

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The toxic feature of two disparate pore-forming toxins, *i.e.*, Cry δ-endotoxins from *Bacillus thuringiensis* (*Bt*, biopesticide) and CyaA-hemolysin (CyaA-Hly) from *Bordetella pertussis* (human pathogen causing whooping cough or pertussis) is generally attributed to their capability to form oligomeric pores, causing lysis of target cells. However, molecular description of their pore-forming process and target specificity has not been clearly defined. Attempts were made to provide more critical insights into membrane-pore formation and receptor recognition for both types of toxins.

For the 65-kDa three-domain *Bt*-Cry4Ba insecticidal protein which is highly toxic to larvae of *Aedes* and *Anopheles* species, vectors of dengue viruses and malaria, respectively, we employed two direct rendering techniques, *i.e.* single-particle negative-stain electron microscopy and high-speed atomic force microscopy. We clearly demonstrated that a membrane-induced state of toxin monomers is a critical prerequisite for the formation of a potential pre-pore trimer. Moreover, the polarity of the Cry4Ba α4-α5 loop residue–$Asn^{166}$ was found to be important for ion permeation and pore-opening. Furthermore, we demonstrated that Cry4Ba utilizes two aromatic loop-residues, $Tyr^{332}$ and $Phe^{364}$ which are respectively located in $\beta_2$-$\beta_3$ and $\beta_4$-$\beta_5$ loops comprising the receptor-binding domain, for synergistic interactions with its alternative receptor–Cyt2Aa2 from *Bt* subsp. *darmstadiensis*. Recently, we have disclosed functional importance of the C-terminal domain of Cry4Ba for serving as membrane- and receptor-binding moiety, indicative of its potential contribution to the toxin bioactivity.

Unlike Cry4Ba, CyaA-Hly requires palmitoylated by CyaC-acyltransferase at $Lys^{983}$ and thus activated its hemolytic activity against target erythrocytes. Our data showed that the $Lys^{983}$-linked palmitoyl group is not directly involved in either binding to erythrocyte membranes or toxin-induced channel conductivity, but rather required for efficient membrane insertion and pore formation of the acylated CyaA-Hly domain. Recently, we have demonstrated for the first time that the N-terminal hydrophobic region of CyaA-Hly is conceivably required for not only membrane-pore formation but also functional association with CyaC-acyltransferase, and hence effective palmitoylation at $Lys^{983}$. Interestingly, we have successfully generated CyaA-specific humanized VH/$V_H$H nanobodies that would have potential applications in developing a novel anti-pertussis agent.

## References

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