

CyaA 353 AGKSLFDDGLGAAPGVPSGRSKFSPDVLVTPASP...GLRRRPSLGAVEKQ  
 LtxA 103 ..ERGLTLFAPQLDKWIQGNK..HLNSVGS.TGNLTKAIKDVQSV  
 PaxA 114 ..ERGLTLFAPQLDKFLQOHS..KISNVVGSSTGDTVNLKLSQAI  
 ApxIIIA 114 ..ERGLTLFAPQLDQFLQHS..KISNVVGSSTGDAVSKLAKSQTI  
 ApxIA 101 ..ERGLTLFAPQLDKLNNK..KLSKSLGSSSEALGQRNLKQTQTA  
 EhxA 90 ..ERGVAIFAPQLDKLQKQY..KVGSKIGGTAENVNNGKAGTV  
 MmxA 104 ..ERGVAIFAPQLDKLQKQY..NIGNKLGSSEALGQRNLKAGTV  
 HlyA 104 ..ERGVITIFAPQLDKLQKQY..KAGNKLGSSEALGQRNLKAGTV  
 MbxA 81 ..QTGIAISATKLEKFLQKHSTNKLAAGLDS.VENIDRKLKASNV  
 RtxA 102 ..QTGVAVSAKKLELQKYSSQLAKSLGS.SANIDSKLTKTNHI  
 ApxIIA 105 ..DRGIVLFAPIQLDKLQK..KIGNTLGS.ASSISQNIKANTV  
 Lkta 102 ..ERGIVLSAPQLDKLQK..KAGQALGS.AESIQNANKAKTV  
 PILkta 102 ..ERGIVLSAPQLDKLQK..KISKAPGS.SENVAKNLGNAQTL

CyaA 401 DSGYDSLDGVGSRFSLGEVSDMAAVEAAELEMTRQVLHAGARQDDAEPGVSGASAHWQG  
 LtxA 144 LGTLQAFINTAFSGMDLDA...LTKARQ...NG..KNVTDVQLAKA...  
 PaxA 156 ISGVQSVLGSVLAGINLNE...AIIISG...GSELELAKA...  
 ApxIIIA 156 ISGIQSVLGTVLAGINLNE...AIIISG...GSELELAKA...  
 ApxIA 143 LSAALQSVLGTALAGMDLDS...LLRRRR...NG..EDVSGSELAKA...  
 EhxA 132 LSAALQSVLGTALAGMDLDE...LLRKRQ...EG..EDISQNDIAKS...  
 MmxA 146 LSTLQSVLGTALAGMDLDE...LLKKQH...KG..EDVSGSELAKA...  
 HlyA 146 LSTLQSVLGTALAGMDLDE...LLKKQH...SG..GNVSGSELAKA...  
 MbxA 124 LSTLQSVLGTALAGMDLDE...LLKKQH...KG..EDVSGSELAKA...  
 RtxA 145 LSTLQSVLGTALAGMDLDS...LVKQG...DASATDLAKA...  
 ApxIIA 146 LGGIQLSVLGTALAGMDLDE...LLQNK...DFNQLELAKA...  
 Lkta 142 LSGIQLSVLGTALAGMDLDE...ALQNN...SNQHALAKA...  
 PILkta 142 LSGIQLSVLGTALAGMDLDE...LLQNK...GSELELAKA...

CyaA 461 RALQGAQAVAAQRVLVHAIALMTQFGRAGSTNTPQEAASLSAAVFGLEASSAVVETVSG  
 LtxA 182 ...SLNLINELIGTISSITN...NVDTEFSKQLNKLGEALGQV..HFGS  
 PaxA 189 ...GVDLASLVLGNIAKGT...TIEAFSEQLQNFVKLVNAK..GLGG  
 ApxIIIA 189 ...GVDLASLVLGNIAKGT...TIEAFSEQLQNFVKLVNAK..GLGG  
 ApxIA 181 ...GVDLAAQLVDNIAATG...TVDAFAEQLGKLGNAISNT..RLSG  
 EhxA 170 ...SIELINQLVDTVSSINS...TVDSFSEQLNQLGSFSSK..RLSS  
 MmxA 184 ...SIELINQLVDTVSSVNN...NIDAFSEQLNKLGLSNTK..HLKG  
 HlyA 184 ...SIELINQLVDTVSSVNN...NIDAFSEQLNKLGLSNTK..HLKG  
 MbxA 158 ...SIDLINEIIGNLSQSTQ...TIEAFSSQLAKLGSISQAK..GFSN  
 RtxA 179 ...SIDLINEIIGNLSQSTQ...TIEAFSSQLAKLGSISQAK..GFSN  
 ApxIIA 180 ...GELTNELVGNIASVQ...TVDAFAEQLSKLGSLLQNVK..GLGG  
 Lkta 175 ...GELTNELVGNIASVQ...TVDAFAEQLSKLGSLLQNVK..GLGG  
 PILkta 175 ...GELTNELVGNIASVQ...TVDAFAEQLSKLGSLLQNVK..GLGG

CyaA 521 FFRGSSRW.AGGFGVAGGAMALGGGIAAAVGAAGMSLT.DDAPAGQKAAAGAEALQLTGC  
 LtxA 223 FGDKLNLP..KLGNLQKGLGALSGLVLSAISAALLANKDADTATKAAAGAEALQLTGC  
 PaxA 230 VGGQQLQHISSGALSGLTGLDIISSLLSGVTAFTLADKNASTSTKVAAGFELSNQVIGG  
 ApxIIIA 230 VGRQLQNISSGALSGLTGLDIISSLLSGVTAFTLADKNASTSTKVAAGFELSNQVIGG  
 ApxIA 221 LASKLNLP..DLSLAGPFDVAVSGILSVSAFSLSNKDDADAGTKAAAGFELSTKILGN  
 EhxA 211 VGGKLNLP..DLGPGDGLDVVSGILSAVSAFSLGNSDAHTGTKAAAGFELTQVLCN  
 MmxA 225 IGNKLNLP..DLGKLGDTISGILSAISAGFSLGNENADGGTKAAAGFELTQVLCN  
 HlyA 224 VGNKLNLP..NLDNIGALDITVSGILSAISAGFSLSNADADTGTKAAAGFELTQVLCN  
 MbxA 199 IGNKLNLP..NFSKTNLGLIITGLLSGISAAGFALADKNASTGKVAAGFELSNQVIGN  
 RtxA 220 LGNKLNLP..NFGKANLALEMISGLLSGISAAGFSLADKNASTKVAAGFELSNQVIGN  
 ApxIIA 221 LSNKLNLP..DLGKALGLDIISSLLSGASAGFSLADKNASTKVAAGFELSNQVIGN  
 Lkta 216 LGDKLNLP..GLDKAGLDIVISGLLSGATAALVADKNASTAKKVAAGFELSNQVIGN  
 PILkta 216 LGDKLNLP..GFSKAGLGLVISGLLSGATAALVADKNASTGRKVAAGFELSNQVIGN

CyaA 579 TVELASSIALALAAARGVTSGLQVAGASAGAAAGALAAALSPEMEIYGLVQOSSHADQLDK  
 LtxA 281 IGAITQYILAQRA...AAGLSTTGPVAGLIASVVSALISPLSFLGIAGKDRARMLEE  
 PaxA 290 ITKAVSSYILAQRL...AAGLSTTGPAAALIASISLAIPLSFLRVADNFRSKDIRE  
 ApxIIIA 290 ITKAVSSYILAQRL...AAGLSTTGPAAALIASISLAIPLSFLRVADNFRSKDIRE  
 ApxIA 279 IGAITQYILAQRA...AAGLSTTAAATGLIGSVVALISPLSFLNVADKFERAKOLEQ  
 EhxA 269 VQKAVSQYILAQRM...AAGLSTTAAAGLITSAVMLAISPLSFLAIADKFERAKOLEQ  
 MmxA 283 VQKAVSQYILAQRV...AAGLSTTAAAGLIASAVVAISPLSFLAVADKFERAKOLEQ  
 HlyA 282 VQKAVSQYILAQRA...AAGLSTTAAAGLIASAVVAISPLSFLAIADKFERAKOLEQ  
 MbxA 256 VTKAISSYVLAQRV...AAGLSTTGAVVALITSSIMLAISPLAFMNAADKFNHNAALDE  
 RtxA 278 VTKAISSYVLAQRA...AAGLSTTGAVVALITSSIMLAISPLAFMNAADKFNHNAALDE  
 ApxIIA 279 VTKAISSYVLAQRV...AAGLSTTGPVAAALIASTVLAISPLSFLNVADKFERAKOLEQ  
 Lkta 274 ITKAVSSYILAQRV...AAGLSTTGPVAAALIASTVLAISPLSFLAIADKFNHNAKLES  
 PILkta 274 ITKAVSSYILAQRV...AAGLSTTGPVAAALIASTVLAISPLSFLAIADKFNHNAKLES

CyaA 639 LAQESSAYGYECDALLAQLYRDKTAAEGAVAGVSAVLSLVGAAVSIAAAASVVGAPVAVV  
 LtxA 337 YSKRFKFGYECDSLLGQFYKNTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL  
 PaxA 346 FAERFKKFGYECDSLLGQFYKNTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL  
 ApxIIIA 346 FAERFKKFGYECDSLLGQFYKNTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIGLL  
 ApxIA 335 YSERFKKFGYECDSLLGQFYKNTGIADAAITINTVLSAIAAGVGAASAGSLVGAPVAA  
 EhxA 325 YSERFKKFGYECDSLLGQFYKNTGIADAAITINTVLSAIAAGVGAASAGSLVGAPISML  
 MmxA 339 YSQRFKKFGYECDSLLGQFYKNTGIADAAITINTVLSAIAAGVGAASAGSLVGAPISAL  
 HlyA 338 YSQRFKKFGYECDSLLGQFYKNTGIADAAITINTVLSAIAAGVGAASAGSLVGAPVSA  
 MbxA 312 FAKQFKKFGYECDSLLGQFYKNTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIAL  
 RtxA 334 YSQRFKKFGYECDSLLGQFYKNTGIADAAITINTVLSAIAAGVGAASAGSLVGAPVALL  
 ApxIIA 335 YSERFKKFGYECDSLLGQFYKNTGIADAAITINTVLSAIAAGVGAASAGSLVGAPVALL  
 Lkta 330 YSERFKKFGYECDSLLGQFYKNTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIAL  
 PILkta 330 YSERFKKFGYECDSLLGQFYKNTGIADAAITINTVLSAIAAGVGAASAGSLVGAPIAL

CyaA 699 TSLTGLANGILRGVQPIIEKLIANDYARKIDELGG...PQAYFEKNLQARHEQLANSNDG  
 LtxA 397 VSAITGLISGILDASKQAVFEHIANQLADKIKAWENK.YGKNYFENGYDARHSAFLE..D  
 PaxA 406 VTGITGLISGILEFSKQPMLEHVASKLGTKEDEWERK.YGKNYFENGYDARHSAFLE..D  
 ApxIIIA 406 VTGITGLISGILEFSKQPMLEHVASKLGTKEDEWERK.YGKNYFENGYDARHSAFLE..D  
 ApxIA 395 VSAITGLISGILDASKQAVFEHIANQLADKIKAWENK.YGKNYFENGYDARHSAFLE..D  
 EhxA 385 VSAITGLISGILEASKQAMFEHVADKFAARINWEWEK.YGKNYFENGYDARHSAFLE..D  
 MmxA 399 VSAITGLISGILEASKQAMFEHVADKFAARINWEWEK.YGKNYFENGYDARHSAFLE..D  
 HlyA 398 VSAITGLISGILEASKQAMFEHVADKFAARINWEWEK.YGKNYFENGYDARHSAFLE..D  
 MbxA 372 VAGVTGLISGILEASKQAMFEHVANRLLQKILEWEKQNGGQYFDKGYDSRYAAYLA..N  
 RtxA 394 VAGVTGLISGILEASKQAMFEHVANRLLQKILEWEKQNGGQYFDKGYDSRYAAYLA..N  
 ApxIIA 395 VAGVTGLITILEYSKQAMFEHVANKVHDRIVWEWK.HNKNYFEGYDSRHLADLQ..D  
 Lkta 390 VSGITGVISTILQYSKQAMFEHVANKIHNKIVWEKNNHGKNYFENGYDARYLANLQ..D  
 PILkta 390 VSGITGVISTILQYSKQAMFEHVANKIHNKIVWEKNNHGKNYFENGYDARYLANLQ..D

**Supplementary Figure S1. Alignment of the pore-forming domain of CyaA against other RTX toxins.** Multiple Sequence Alignment (MSA) was performed with Clustal Omega software and plotted with ESPript 3.0. The name of the toxin and the number of the corresponding residues are shown in the left side. The strictly conserved alanine, arginine, aspartic acid, glutamic acid, glutamine, glycine, isoleucine, leucine, lysine, proline, threonine and tyrosine residues are highlighted with a red frame. CRAC motifs are depicted in purple and CARC motifs in blue.