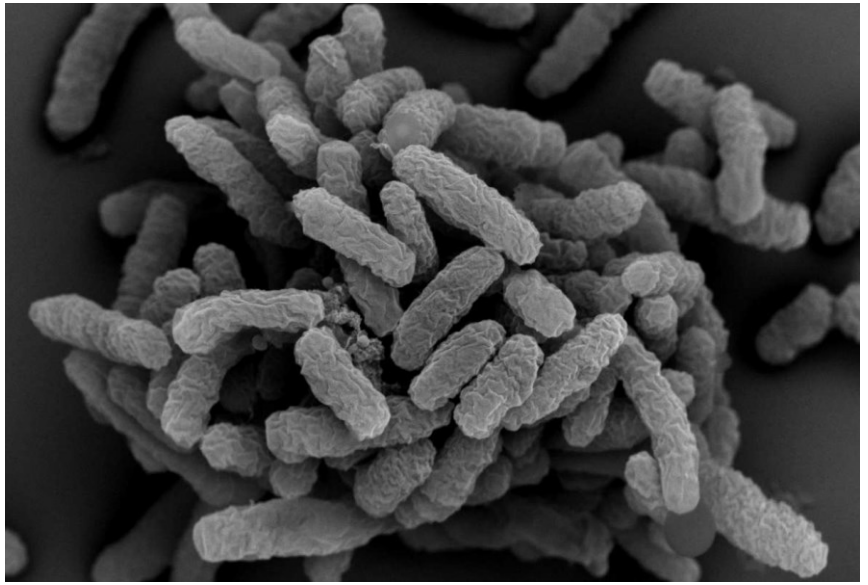
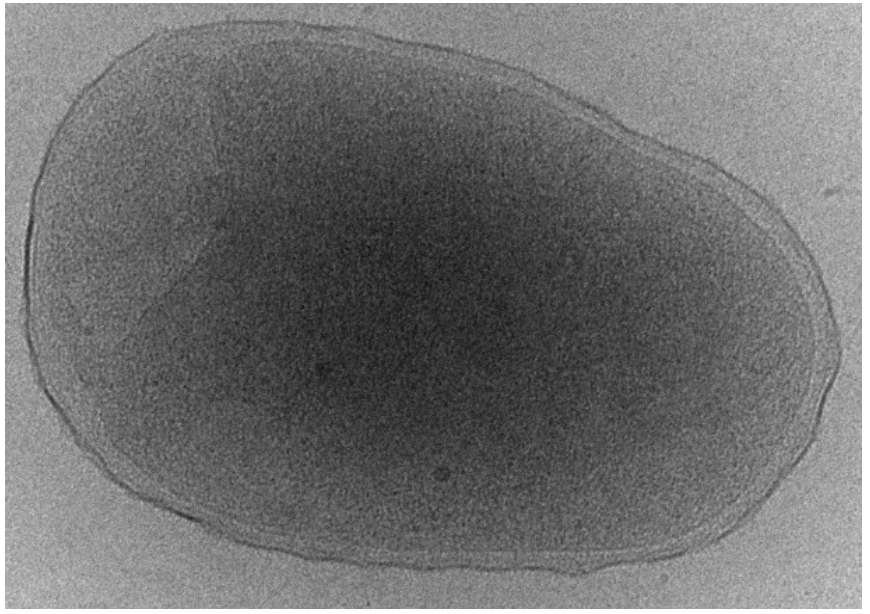
Acylation dictates the extent of activation of bacterial RTX toxins

David Jurnečka

Bordetella pertussis

- exclusively human pathogen and the causative agent of whooping cough
- one of the world least controlled vaccine-preventable diseases
- accounting for more than 40 million cases and close to 200,000 deaths every year
- severe symptoms are common in young unvaccinated infants
- whole-cell vaccine introduced in 1950s
- exchanged by acellular vaccine in most of the developed countries in early 2000s
    - associated with waning immunity of vaccinated individuals linked to pertussis resurgence
    - contains up to 5 purified antigens



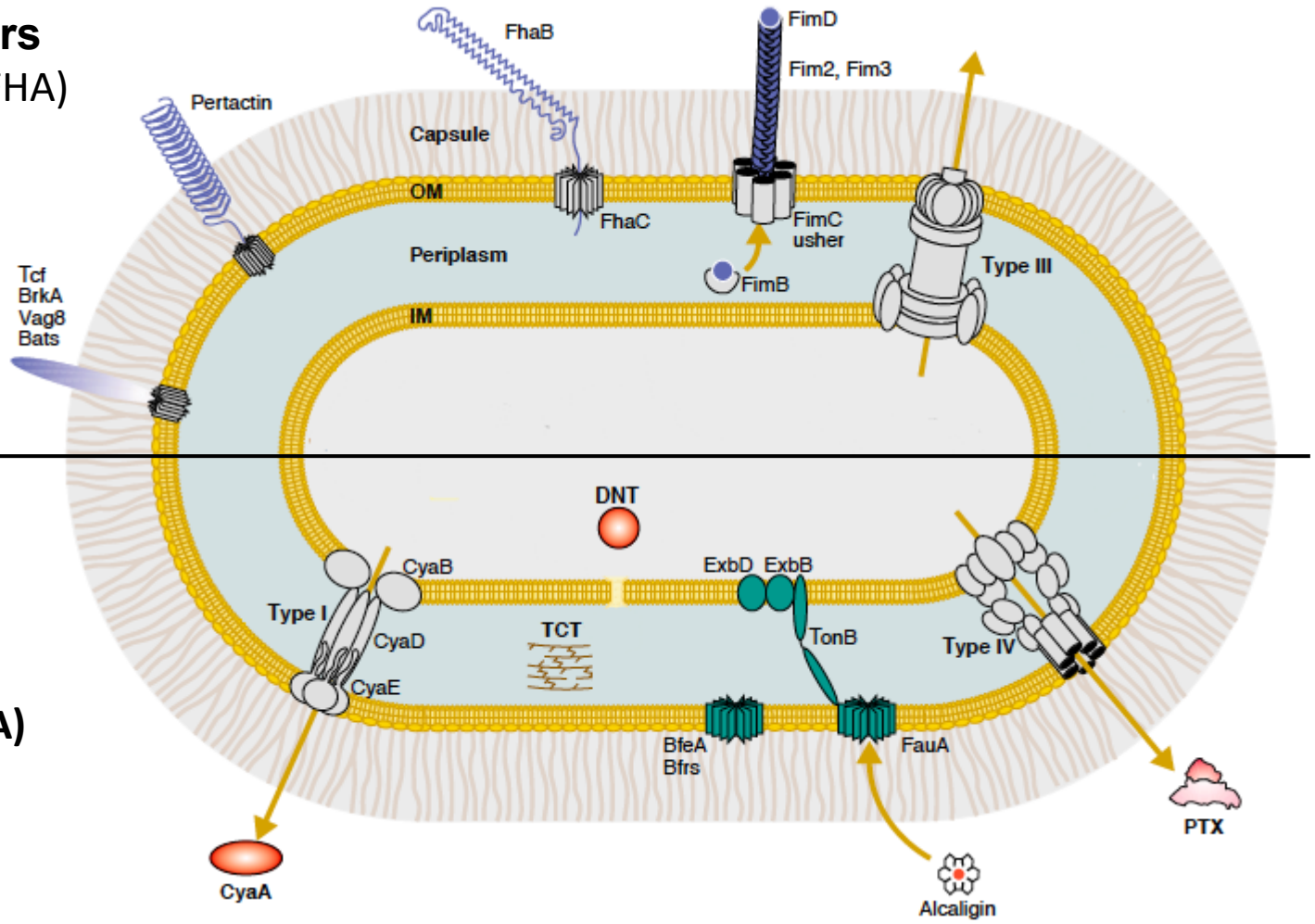


(SEM-Benada O., TEM-Nováček J.)

Virulence factors of *Bordetellae*

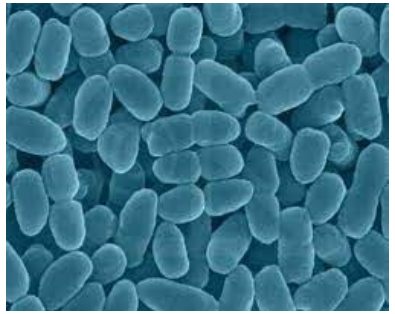
Adhesins/Autotransporters
Filamentous hemagglutinin (FHA)
Fimbriae (Fim2, Fim3)
Pertactin
BrkA
Vag8

Toxins
Pertussis toxin (PTX)
Adenylate cyclase toxin (CyaA)
Tracheal cytotoxin (TCT)
Dermonecrotic toxin (DNT)
Endotoxin (LOS)

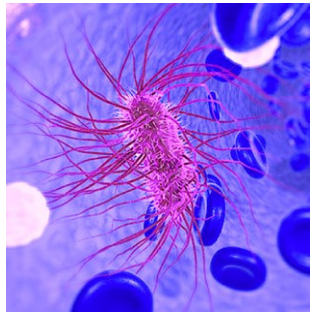


(adapted from Carrof et al, 1990)

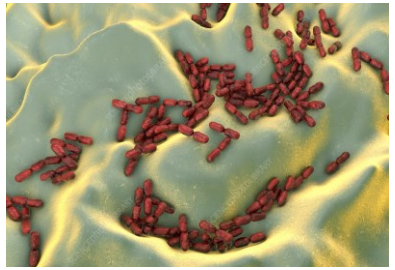
Repeats in ToXin (RTX) cytolysins from various bacterial species



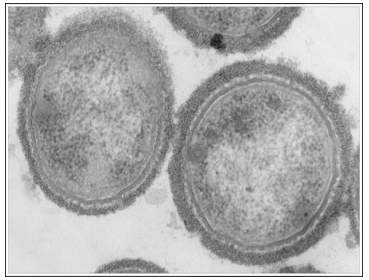
Bordetellae
CyaA



Uropathogenic *Escherichia coli*
HlyA

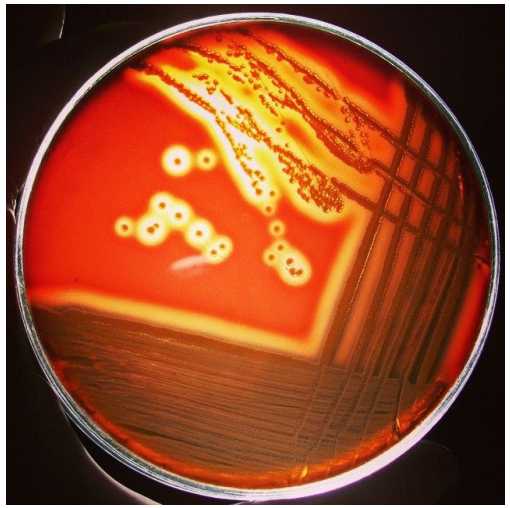


Kingella kingae
RtxA



Actinobacillus pleuropneumoniae
ApxI-IVA

Vibrio, Aggregatibacter, Morganella . . .



Overall characteristics of RTX toxins

```
CyaA 850-EEQRRRTKTGKSEFTTFVEIV-870 ... 973-GVATQTTAYGKRTENVQYRHV-993
HlyA 554-EEIRERRQSGKYEYITELLVK-574 ... 680-VVKEQEVSVGKRTEKTQYRSY-700
RtxA 548-TETRERVQNGKYSYINQLKFN-568 ... 679-VIKRQTTQVGKRTETLEYRDF-699
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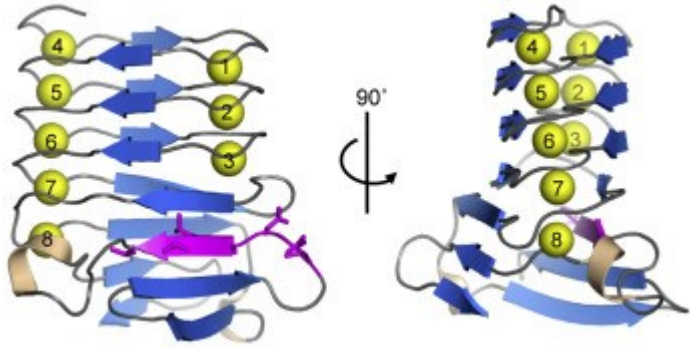
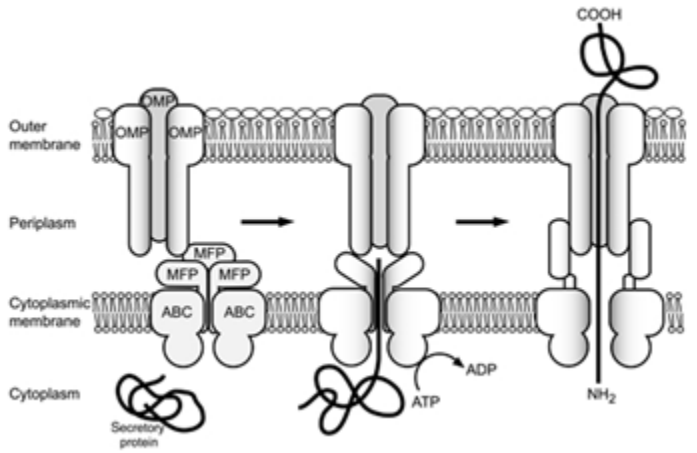
N-terminal pore-forming hydrophobic domain

central segment harboring post-translationally acylated lysine residues activated by a cognate acyltransferase

Ca^{2+}-binding domain comprising tandem gly- and asp-rich nonapeptide repeats

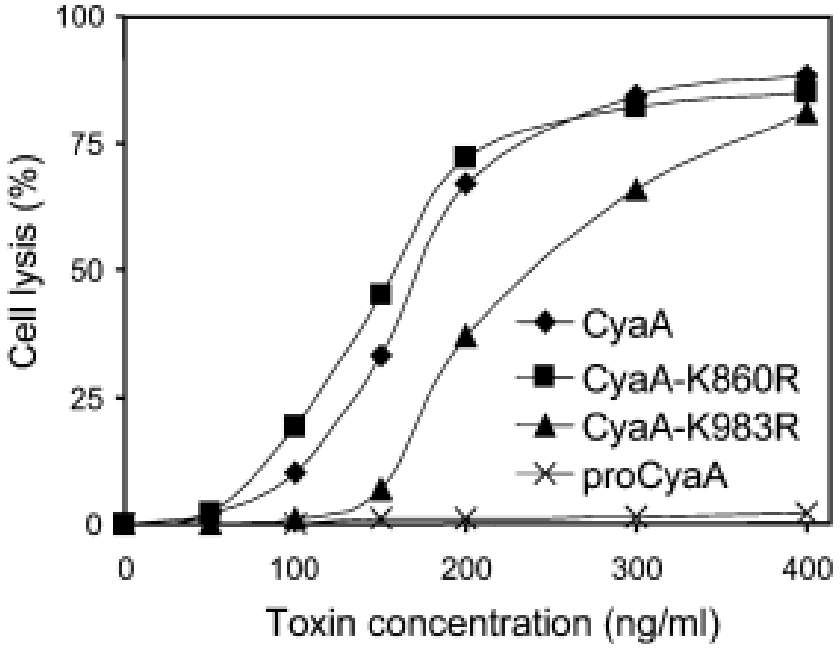


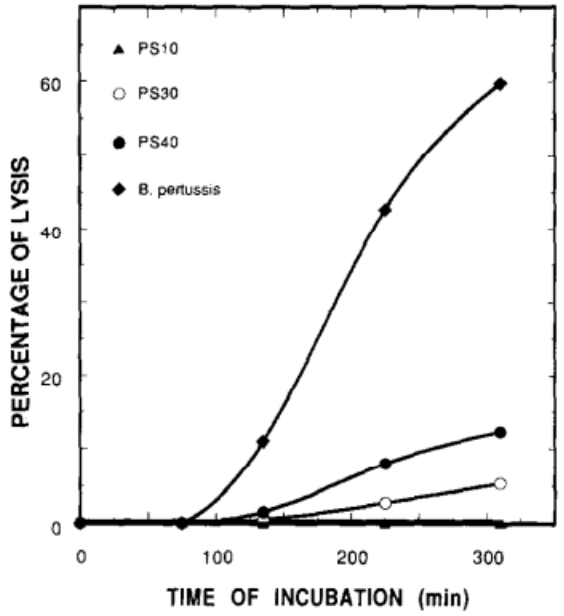


non-cleavable C-terminal secretion signal for the Type I secretion system (T1SS)

(adapted from Bumba et al., 2016; Linhartova et al., 2010)

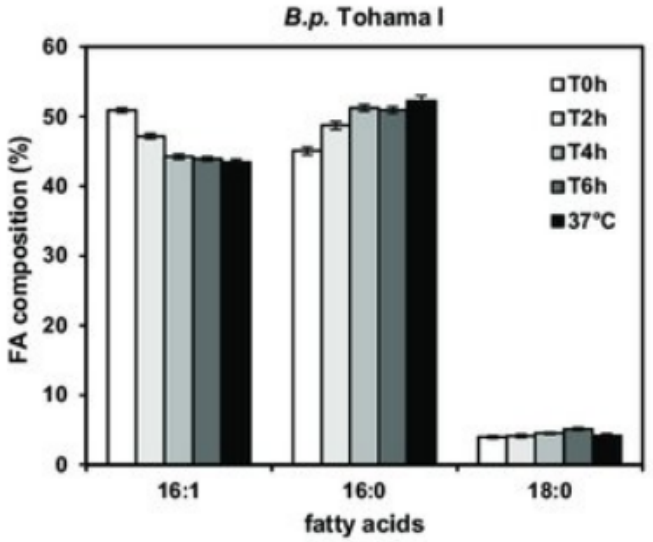
Biological activity of RTX toxins depends on their acylation status





Bp CyaA-C16:0

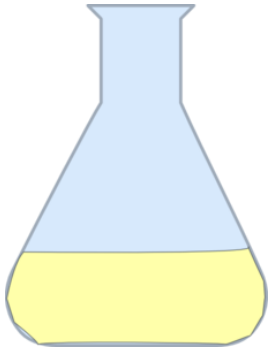
recombinant CyaA-C16:0/C16:1



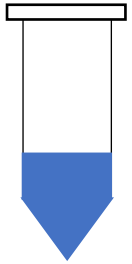
(Sebo et al., 1999; Basar et al., 2001, Masin et al. 2005)

Strategy for purification of recombinant RTX toxins

Transformed *E. coli* BL21/pMM100
cultured at 37°C in MDO medium
3h post IPTG induction

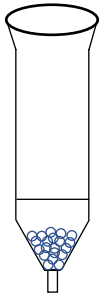


harvesting
sonication
centrifugation

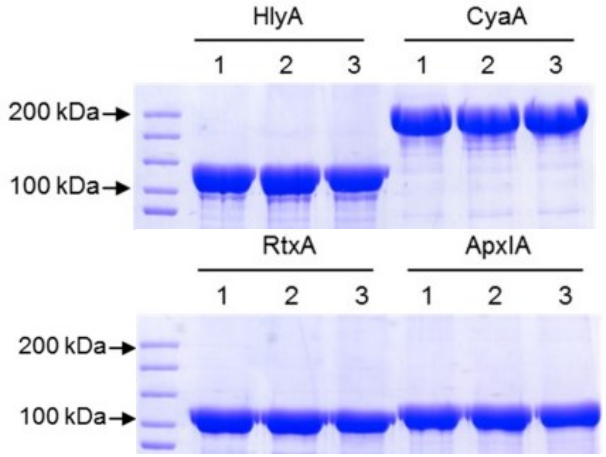


The cleared urea extracts
of inclusion bodies

load at room temperature



Affinity chromatography - **Ni-NTA agarose**
Ion Exchange chromatography – **DEAE sepharose**

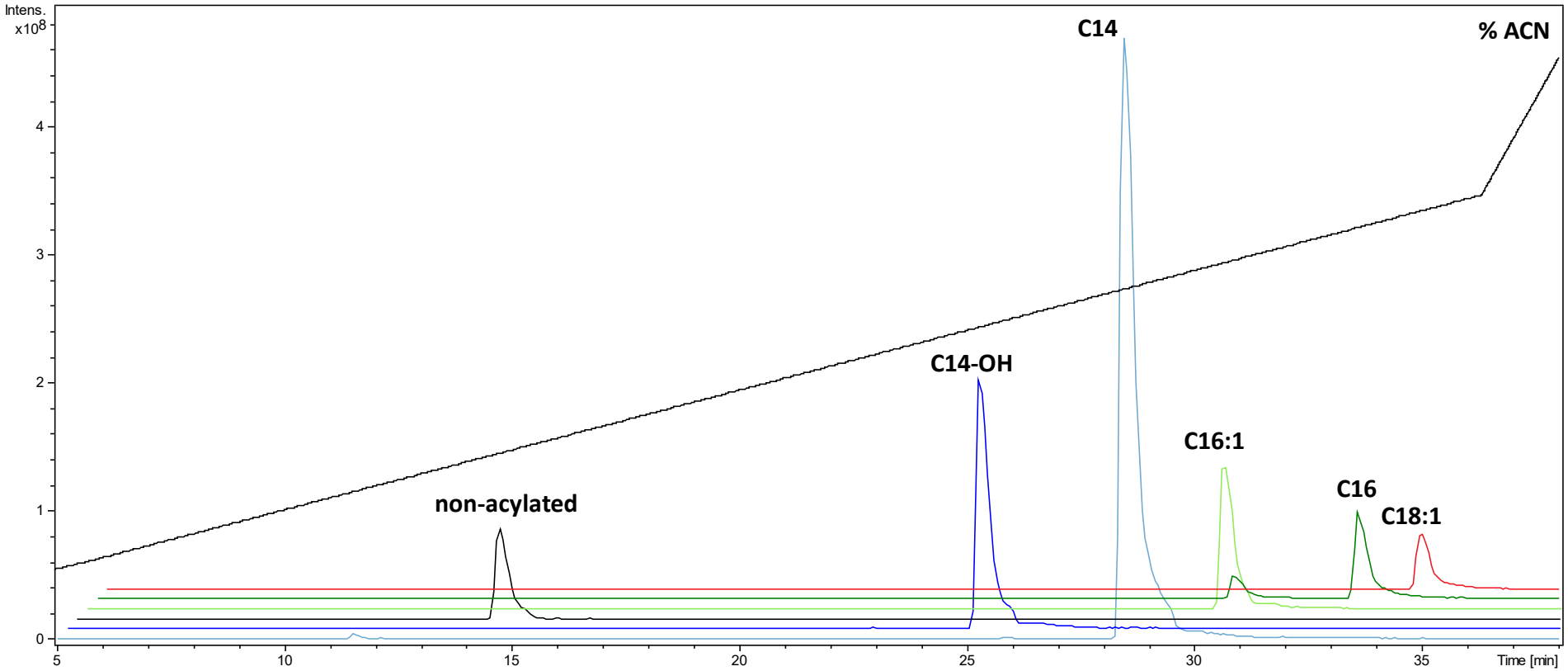


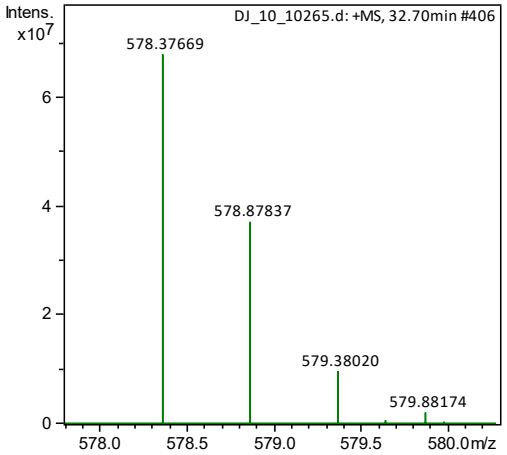
dilution in 50 mM ammonium bicarbonate buffer (pH 8.2) to reach 4 M urea concentration

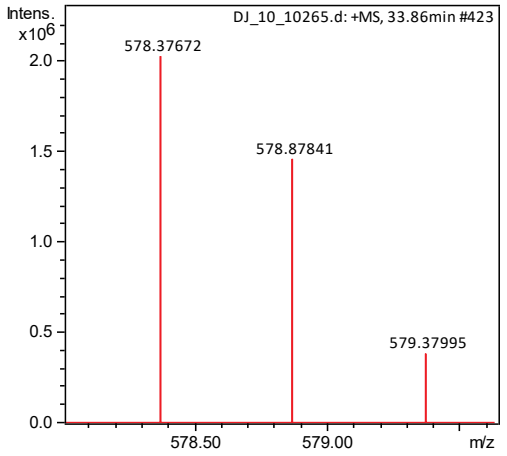
Trypsin
30°C
12h

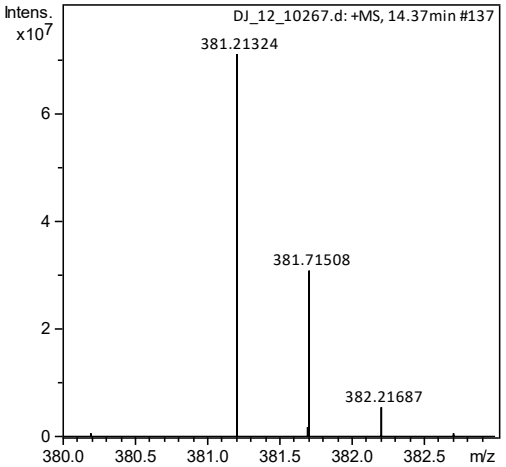
LC system
desalting and separation

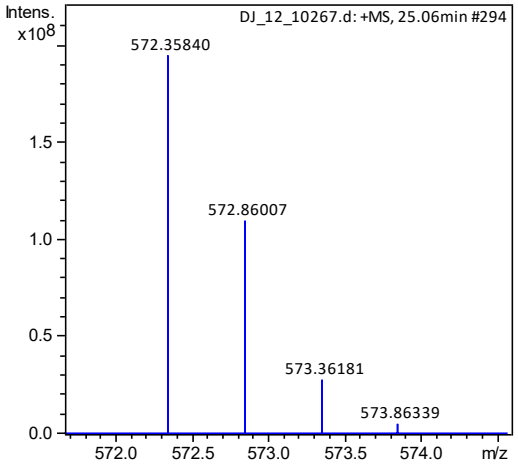
Acylation of RtxA peptide QTTQVGKR

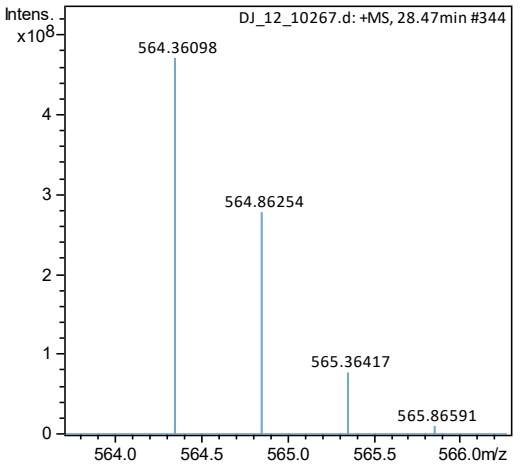


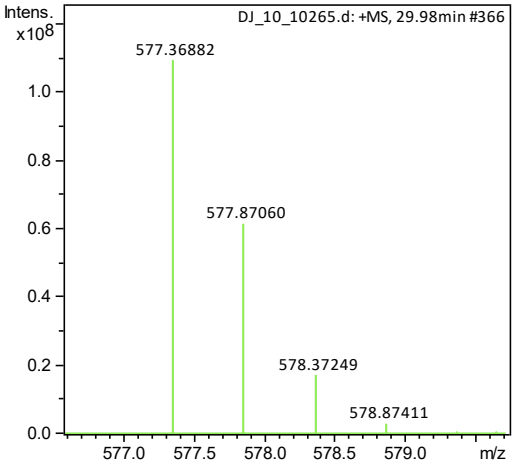






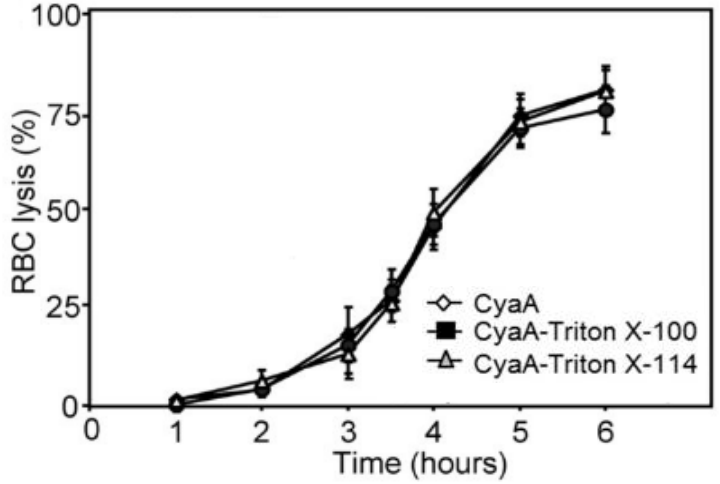


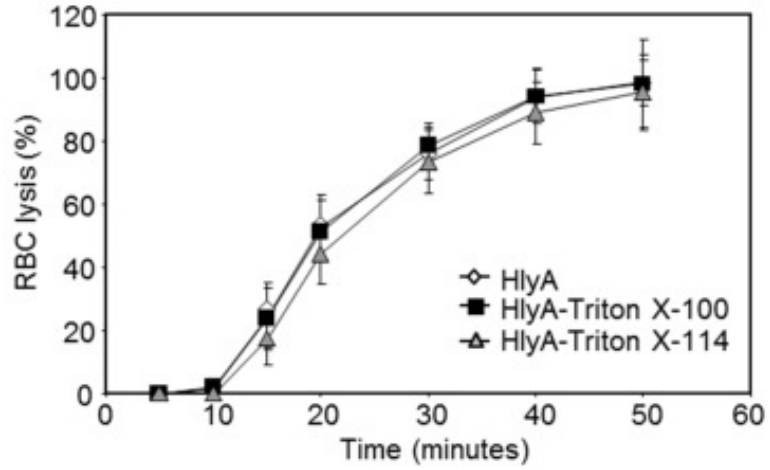


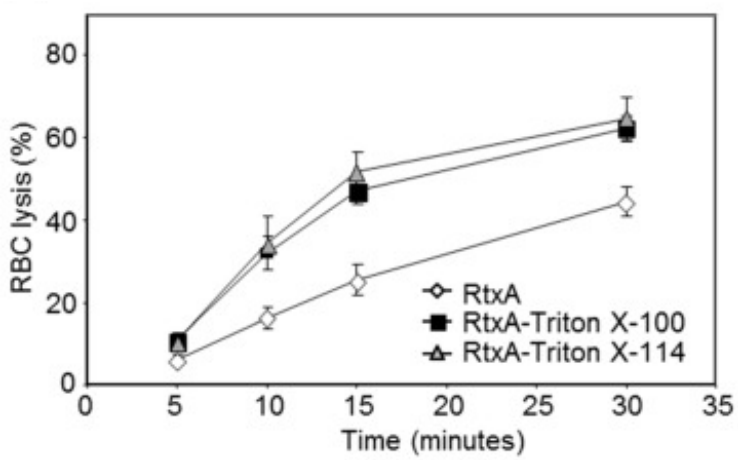


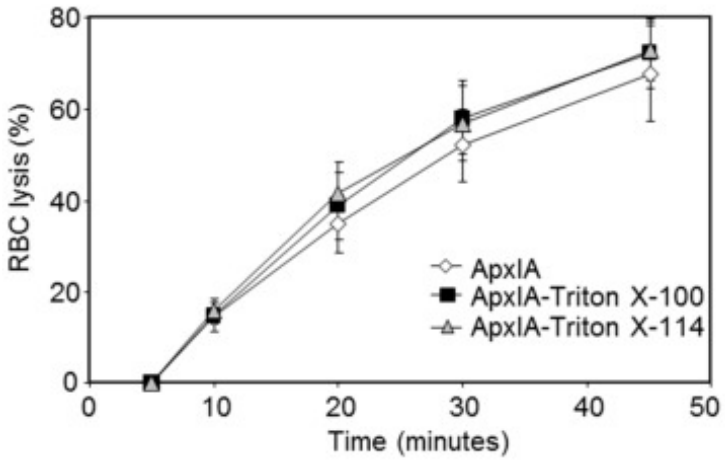
Acylation status and cytolytic activity of RTX toxins activated by cognate acyltransferases

Protein	1st Lysine	2nd Lysine
CyaA	C16:0/C16:1	C16:0/C16:1
HlyA	C14:0/C14:0-OH	C14:0/C14:0-OH
RtxA	-	C14:0/C14:0-OH
ApxIA	C14:0/C14:0-OH	C14:0/C14:0-OH



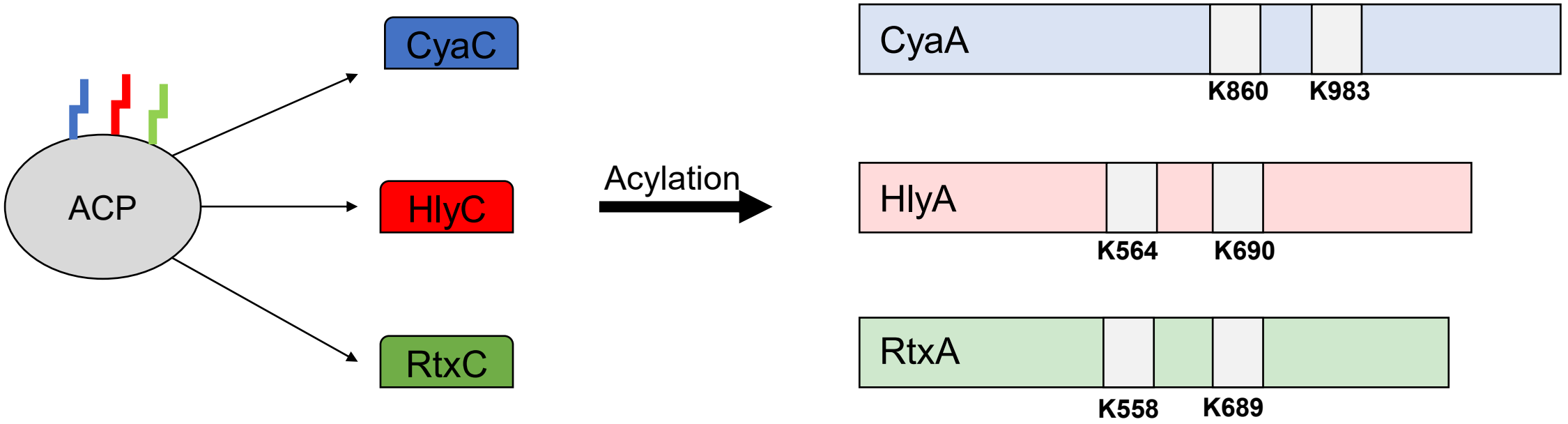






Determination of acylation status of RTX toxins

Acyltransferase		RTX toxin
CyaC		CyaA
HlyC	X	HlyA
RtxC		RtxA



(Osickova et al., 2020)

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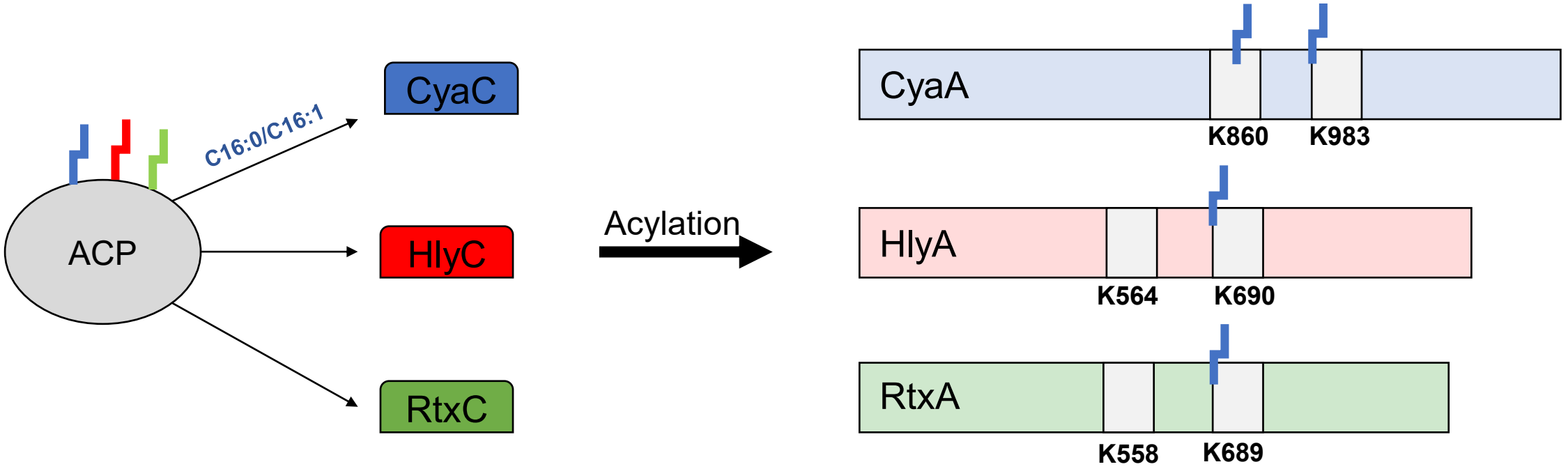

(Osickova et al., 2020)

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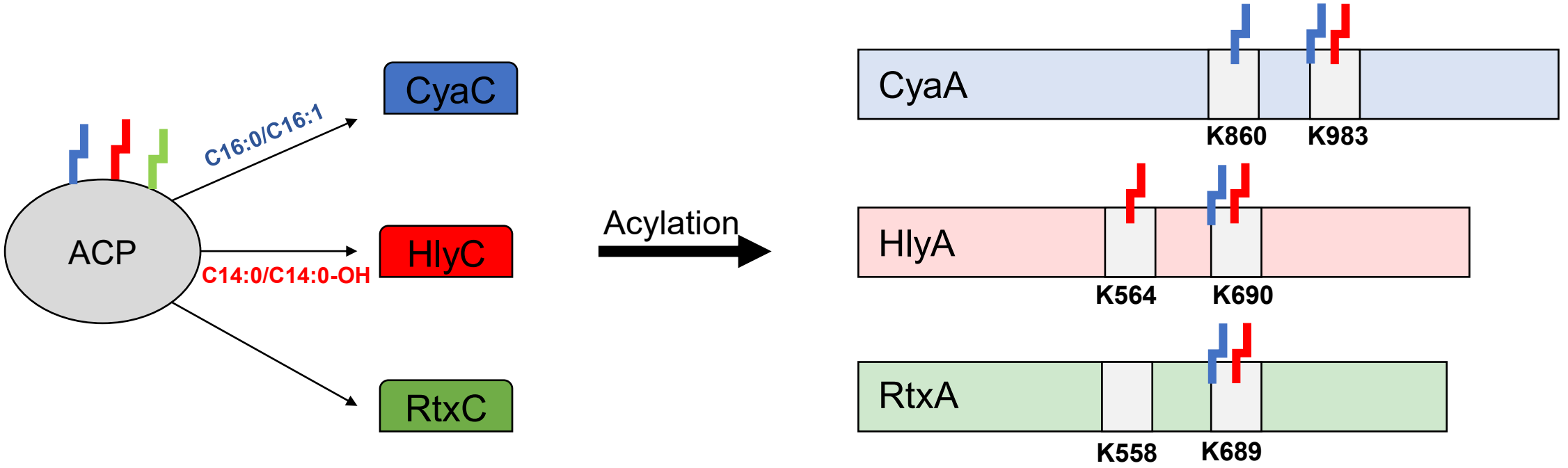

(Osickova et al., 2020)

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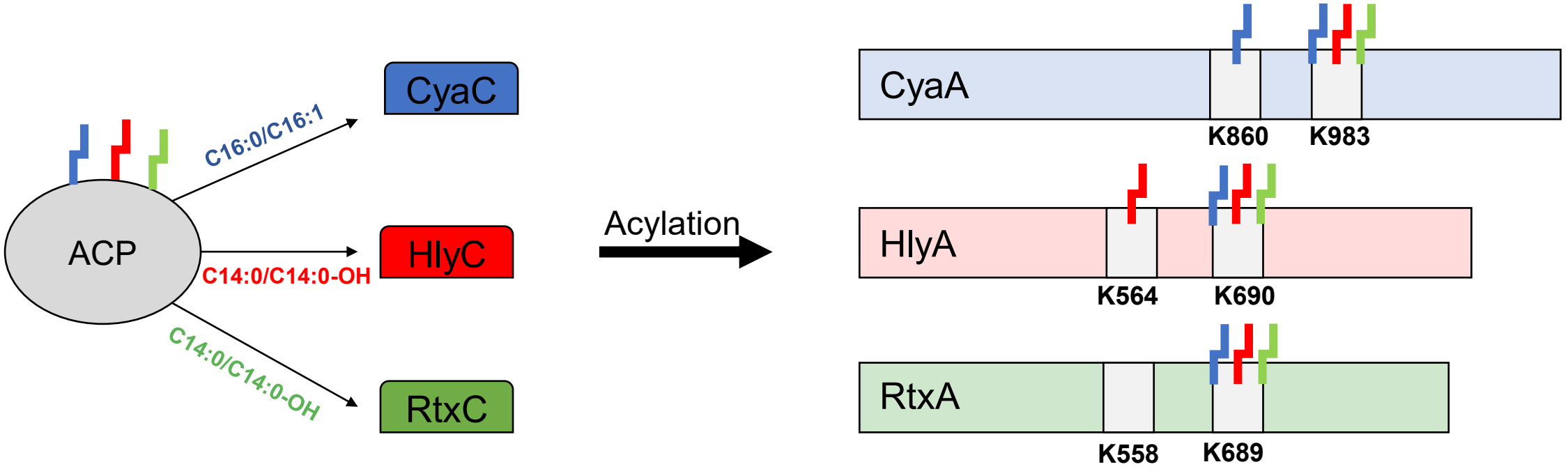
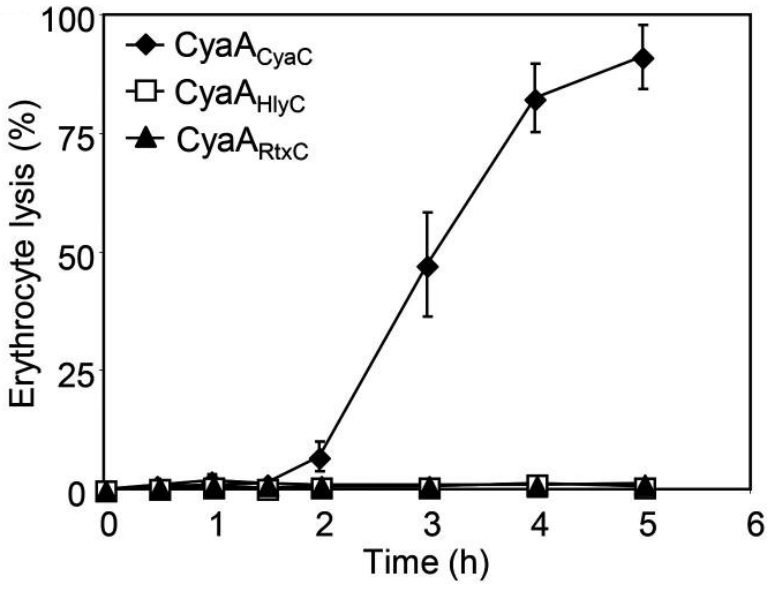


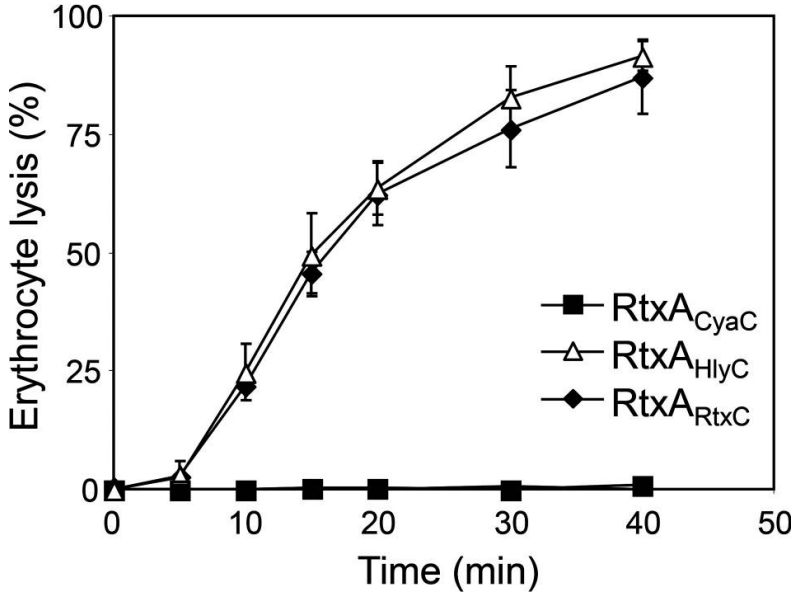
(Osickova et al., 2020)

Biological activity of differently acylated RTXA toxins

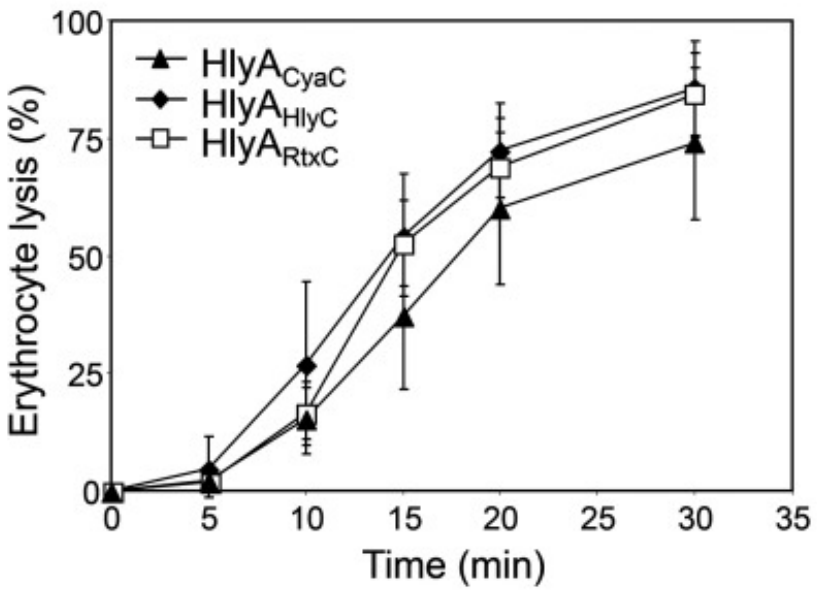
CyaA
C16/C16:1



RtxA
C14/C14:0-OH



HlyA
C16/C16:1
C14/C14:0-OH



(Osickova et al., 2020)

EU FT-ICR MS

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Thank you for your attention !