# First Canadian Peptide and Protein Community Virtual Symposium

# Antibacterial activity of desCys$^{11}$/Lys$^{12}$/Lys$^{13}$-(p-BthTX-I)$_2$K conjugated to cell-penetrating peptides

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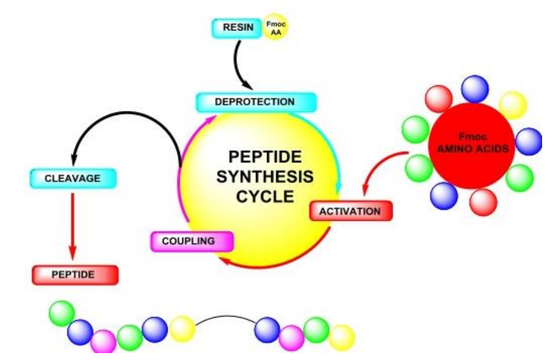
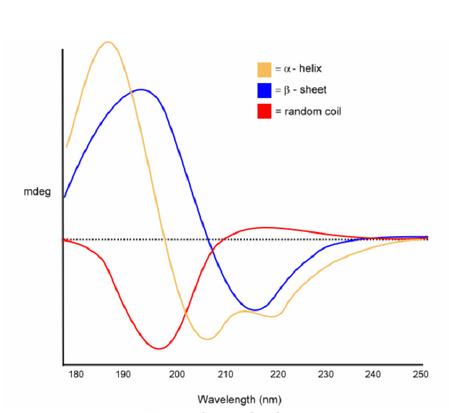
## INTRODUCTION

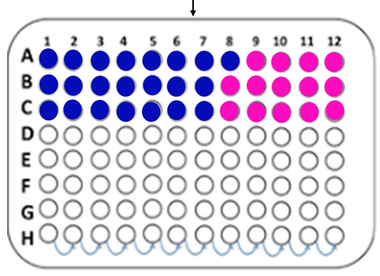
Bacterial pathogen resistance has become a global problem, reaching alarming levels (WHO, 2019). Antimicrobial peptides (AMPs) have been evaluated as an alternative treatment for bacterial infections (GRECO et al., 2020). The peptide (desCys$^{11}$/Lys$^{12}$/Lys$^{13}$(p-BthTX-I)$_2$K), is an example that demonstrates high activity against bacterial strains (SANTOS-FILHO al., 2017). The use of new molecules for drug delivery can be used for the delivery of AMPs, maintaining their activity on specific targets, increasing half-life time and decreasing toxic effects (SANTOS-FILHO et al., 2021). Cell penetrating peptides (CPPs) are molecules capable of crossing biological membranes through energy-independent or energy-dependent processes without destroying membrane integrity in a non-invasive manner (NEUNDORF, 2019; BÖHMOVÁ et al., 2018; RUSESKA; ZIMMER, 2020). The objective of this study was to evaluate the effect of the conjugation of the peptide (desCys$^{11}$/Lys$^{12}$/Lys$^{13}$(p-BthTX-I)$_2$K with PPCs AIP-6, PFV and HIV-TAT (47-57) in terms of structure, biological activity and toxicity.

## MATERIALS AND METHODS

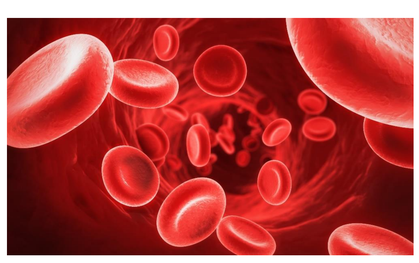



Circular dichroism spectroscopy




Antimicrobial activity

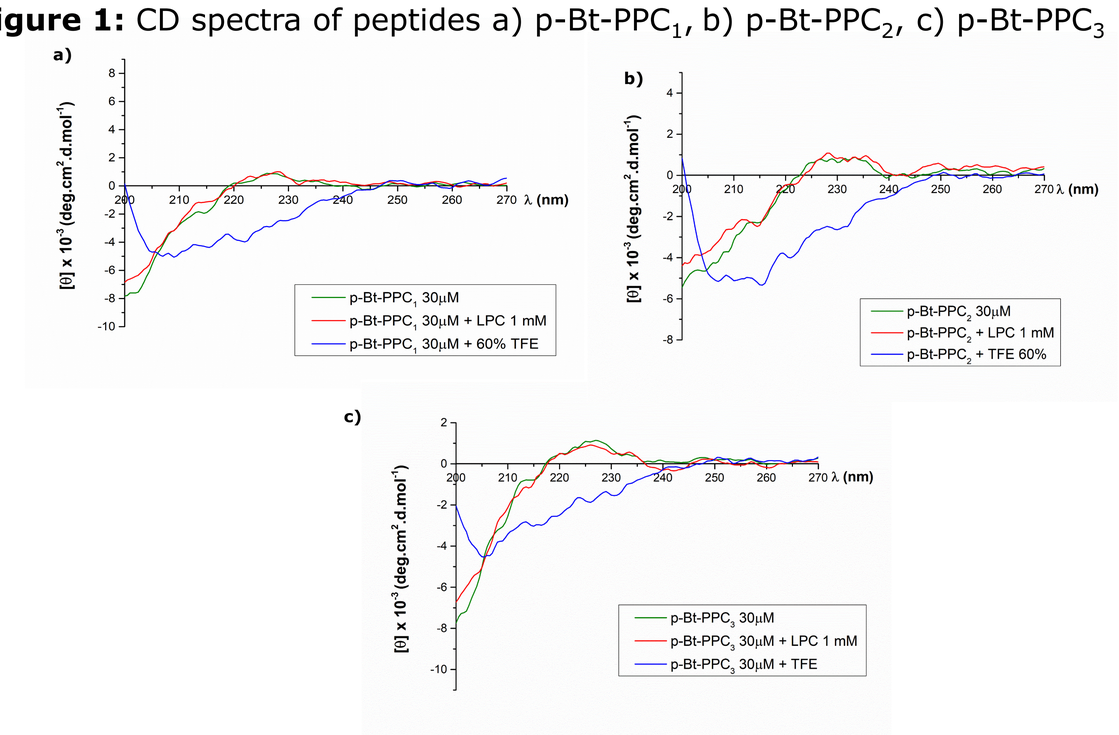


Hemolytic activity

## RESULTS

### Circular dichroism spectroscopy

**Figure 1:** CD spectra of peptides a) p-Bt-PPC$_1$, b) p-Bt-PPC$_2$, c) p-Bt-PPC$_3$




### Antimicrobial activity

**Table 1:** Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) in μM.

| Peptides | *E. coli* ATCC 10536 | | *S. aureus* ATCC 14458 | |
|---|---|---|---|---|
| | MIC | MBC | MIC | MBC |
| p-Bt | 4 | 4 | 128 | 256 |
| PPC$_1$ | 128 | 512 | >512 | >512 |
| PPC$_2$ | 512 | >512 | 512 | >512 |
| PPC$_3$ | 128 | 128 | 128 | 128 |
| p-Bt-PPC$_1$ | 4 | 4 | 8 | 8 |
| p-Bt -PPC$_2$ | 4 | 8 | 16 | 16 |
| p-Bt-PPC$_3$ | 4 | 4 | 16 | 16 |
| Ciprofloxacin | 0.078 | 0.0315 | 2 | 4 |

### Hemolitic activity

| Peptides | % Hemolisys |
|---|---|
| **p-Bt** | 0 |
| **p-Bt-PPC$_1$** | 0 |
| **p-Bt-PPC$_2$** | 0 |
| **p-Bt-PPC$_3$** | 28 |

## CONCLUSION

- The solid-phase peptide methodology was efficient in the synthesis of conjugated peptides.
- The peptides did not show α-helical structure in membrane-mimetic environment (LPC micelles).
- The peptides derived from the conjugation of p-Bt with PPCs showed promising results against Gram-positive bacteria, such as *S. aureus*. Highlight for the p-Bt-PPC1 peptide, which showed the lowest MIC.
- The conjugation with the PPCs did not show hemolytic activity, except for the p-Bt-PPC$_2$ peptide, even with this result the peptide did not reach a HC50, still proving advantageous for its use.
- We demonstrated that (pBthTX-I)$_2$ analogs are promising prototypes for the treatment of infections caused by Gram-negative and Gram-positive bacteria.

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