A robust platform for recombinant production of animal venom toxin modulators of ion channels

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Medical Research Council

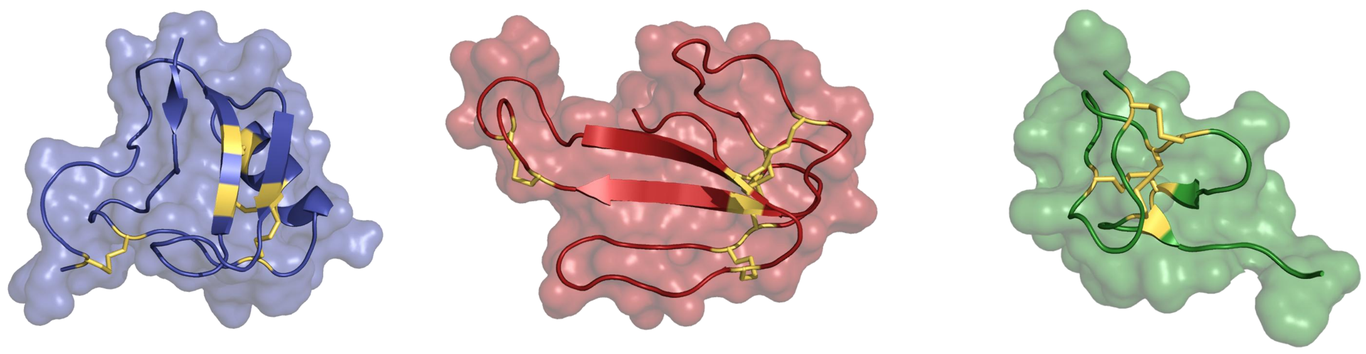
INTRODUCTION

Due to their size, structural diversity and evolutionary selection, toxins are vital for ion channel drug discovery efforts; often serving as exploratory compounds and lead molecules.

Recombinant production would enable faster SAR campaigns of toxins and enable the production of toxin libraries for screening.

Toxins are unable to be produced recombinantly due to 'disulfide scrambling' resulting in peptides with poor stability and solubility.

By attaching an Fc protein as a carrier protein[1], we aim to improve the recombinant yield and improve *in vivo* pharmacokinetic stability and functionality.



AaHII: VKDGYIVDDVVCTYFCGRNAYCNEECTKLKGESGYCQWASPYGNACYCYKLPDHVRTKGPGRCH

αCBTx: IRCFITPDITSKDCPNGHVCYTKTWCDAFCSIRGKRVDLGCAATCPTVKTGVDIQCCSTDNCNPFPTRKRP

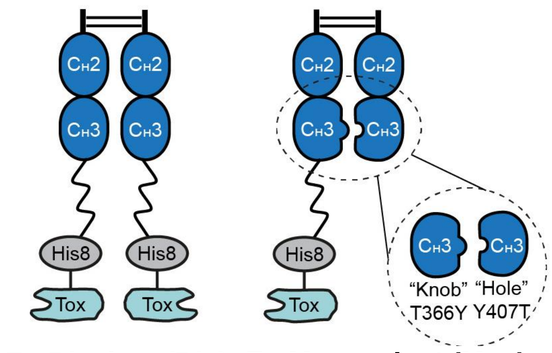
ProTxII: YCQKWMWTCDSERKCCEGMVCRLWCKKKLW — Disulfide bond

Aims

- **Determine if Fc-Toxin fusion improves recombinant peptide yield.**
- **Characterise the potency of the Fc-Toxin peptides at their endogenous ion channel targets.**
- **Determine if the Fc domain can be used for ion channel staining in cells expressing the toxin's ion channel target.**

METHODOLOGY

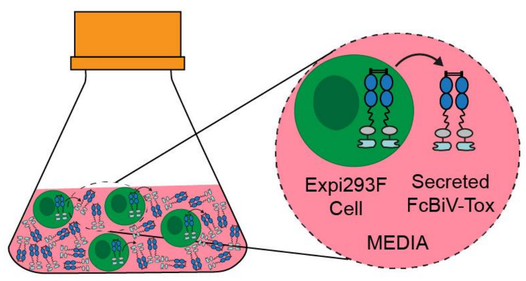
Fc-toxin construct design



Fc-Bivalent (Biv) Fc-Movovalent (Mov)

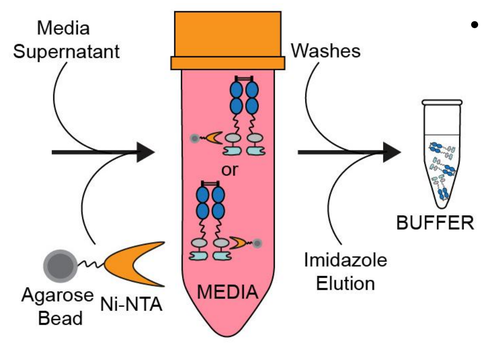
- The toxin was fused to the C-terminus of the toxin, as structural data indicated the C-terminus of the toxins are crucial for binding.
- 'Knob-in-hole'[2] mutation was used to form monovalent Fcs.
- Point mutations (L234F, L235E, P331S) used to attenuate Fc-receptor functions.

Recombinant production



- Expi293F cells transfected at 4 million cells/mL.
- Cells left to secrete the toxin into the media for 5-6 days prior to centrifugation.

Affinity purification



- The his-tag binds to Ni-agarose beads, enabling wash steps prior to elution with imidazole.

RESULTS

1. FcMov-fusion enabled high-yield toxin production

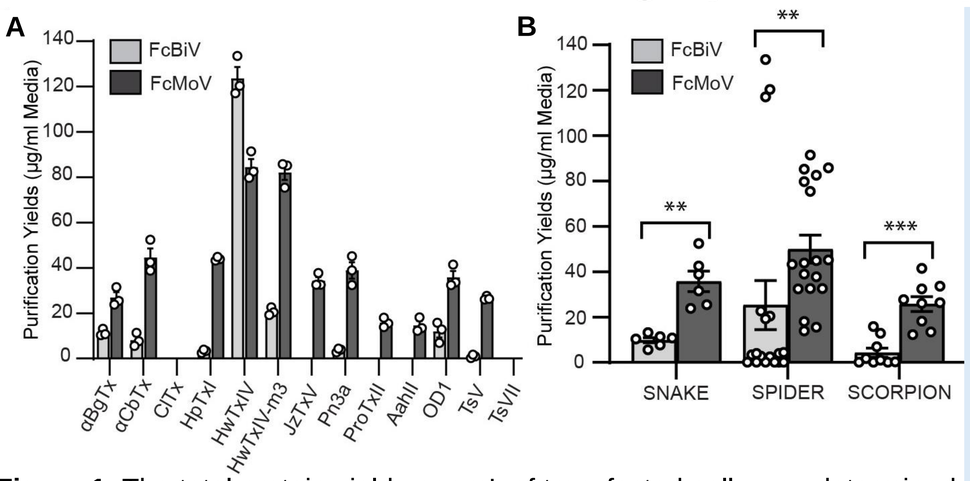


Figure 1. The total protein yields per mL of transfected cells was determined by nanodrop spectrophotometry. **A)** Individual yields of all toxins. **B)** Yield by species of origin.

Of the 13-toxins:

- Only 3 non-Fc toxins were visible on SDS page (data not shown).
- 8 Biv-Fc were able to be affinity purified.
- 11 Mov-Fc were able to be affinity purified (with increased yield.)

2. Fc-Snake toxins retain significant potency

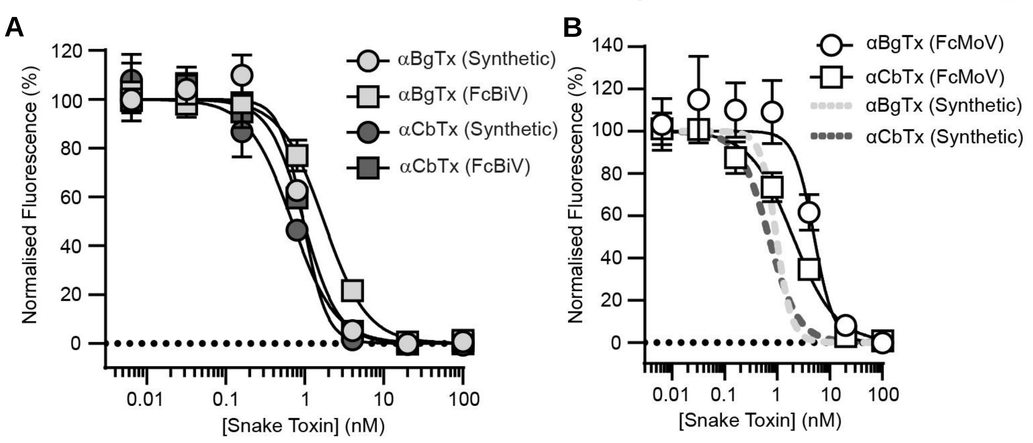


Figure 2. Dose-dependent inhibition by synthetic and FcBiv (A) or FcMov (B) αBgTx and αCBTx snake toxins on fura-2 fluorescent signal generated from the action of 10 µM acetylcholine on nAChR-evoked Ca2+ flux in modified TE671 cells.

- FcBiv-toxins had < 2-fold loss in potency relative to the synthetic toxin.
- Fc Mov-toxins remained within 4-5-fold-loss of potency.

3. Fc-ProTxII more effective than commercial fluorescent toxin for staining

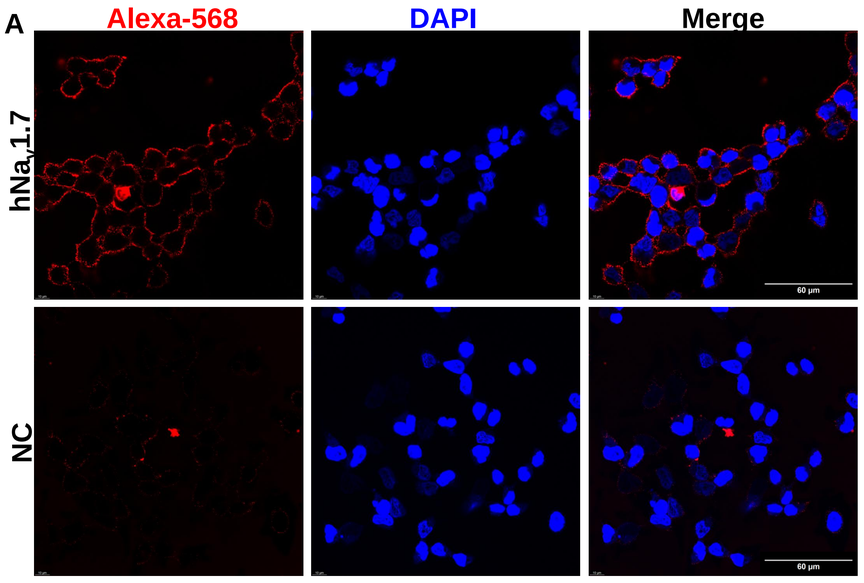


Figure 3. HEK293 cells stably expressing $hNa_v1.7$[3] or HEK293T cells (NC) were incubated with FcProTxII (10 µM) or Atto488 ProTxII[4] (0.1-1 µM). Cells incubated with FcProTxII underwent secondary staining with goat anti-Human Fc (Alexa 568), and tertiary with Donkey anti-Goat Fc (Alexa 568) before fixing and mounting. (A) FcProTxII samples were imaged using confocal microscopy. (B) 150 regions of interest were selected across at least 3 images and the mean grey value (intensity) being extracted. The background was then removed from these values, before a final average was taken.

Summary

- **Fc-Toxin fusion enabled the high-yield production of almost all of the 13 toxins studied, compared to almost no production of the toxins without it.**
- **Only a small loss of potency was observed for the Fc-Snake toxins.**
- **The Fc domain was successfully used to stain cells, demonstrating the added functionality provided by the Fc-domain. This could be used *in vivo* to measure toxin plasma concentrations via ELISA.**

References

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