Validation of antibody toxin fusions against sodium channels

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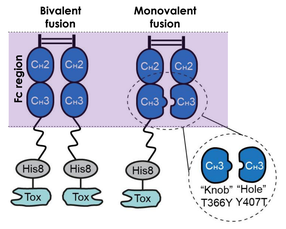
Introduction

- Toxin-derived peptides provide highly selective and potent ion channel modulators
- Generating these peptides is a challenge due to their disulphide arrangements
- Recombinant expression of toxin peptides fused to a scaffold protein is a strategy to overcome these production challenges **(1,2)**
- Effective toxin peptide production could advance both basic research and scaffold-based drug discovery

Aims

- Ten $Na_V1.7$-selective arachnid peptide toxin peptides, fused to the C-terminus (Fc region) of human IgG1, were produced by our collaborators at the Miller lab (University of Cambridge, UK) (see schematic) **(3)**
- Using automated patch clamp technology and neuronal current clamp, we evaluated the potency and selectivity of these Fc-toxin peptide fusions to determine the viability of this approach to produce functional toxin peptides



Toxin peptides ("Tox") were fused to the Fc region of hIgG1 in monovalent form, except for HwTx-IV, which was also produced in bivalent form. Adapted from **(3)**

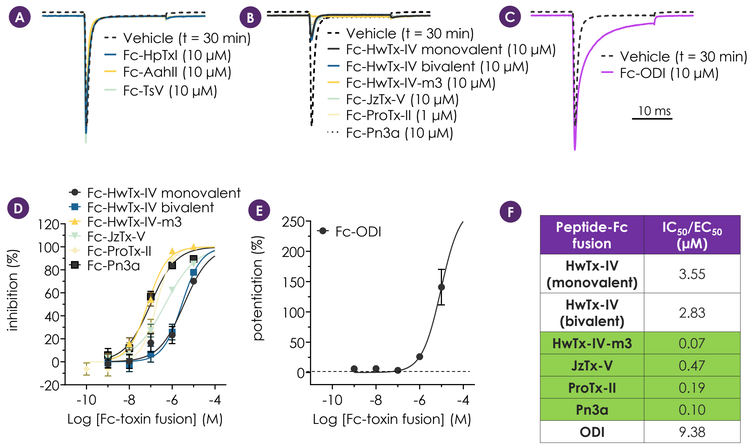
Methods

- **Automated patch clamp electrophysiology** – CHO-hNav1.x cells were held at $V_{0.5}$ inactivation for 8 s before a -10 mV step (0.033 Hz), using a Qube384 (Sophion Bioscience). $V_{0.5}$ was determined for each well, using the adaptive protocol feature. Peptide was applied for 30 min. Inhibition and potentiation are defined as decrease in current amplitude and increase in charge at -10 mV, respectively. Concentration-response graphs displayed as Mean ± SEM, n = 3 – 11/concentration.
- **Rat dorsal root ganglion current clamp (Manual patch clamp)** – Neurons were isolated from 21 – 27 day old male Wister Han rats. Neurons were clamped at -70 mV and subject to an ascending ramp of current injection (500 ms, 0.1 Hz). Ramp amplitude was adjusted for each neuron to comparable multiple firing between neurons at baseline.

Results

1. Screening of Fc-toxin peptide fusions against $Na_V1.7$

Fc-toxin fusions were screened against $Na_V1.7$. Three of the fusions were inactive at the top concentration (A), whereas six demonstrated inhibitory activity (B,D) and one (ODI) potentiated $Na_V1.7$ charge (C,E). The four most potent fusions, all inhibitors – HwTx-IV-m3, JzTx-V, ProTx-II and Pn3a, were selected for further screening (F).

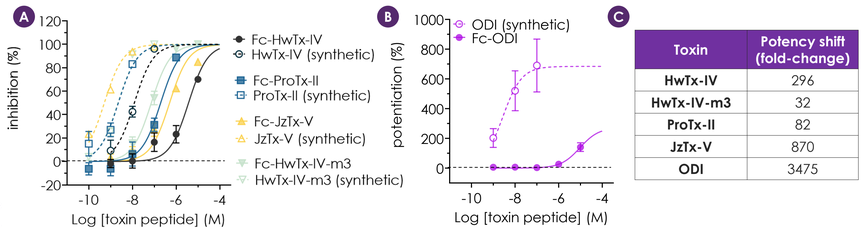


F

Peptide-Fc fusion	IC_{50}/EC_{50} (µM)
HwTx-IV (monovalent)	3.55
HwTx-IV (bivalent)	2.83
HwTx-IV-m3	0.07
JzTx-V	0.47
ProTx-II	0.19
Pn3a	0.10
ODI	9.38

2. $Na_V1.7$ potency comparison between synthetic and Fc-toxin peptides

Fc-toxin peptide activity against $Na_V1.7$ was benchmarked against their synthetic counterpart for the peptides which are commercially available (A,B). A loss of potency is common for recombinant, cysteine-rich peptides due to reduced folding success and lack of possible post-translational modifications. A 50-fold increase in IC_{50} has been reported for SUMO-HwTx-IV previously **(1)**, which aligns with what was observed for Fc-fused HwTx-IV, HwTx-IV-m3 and ProTx-II (C). JzTx-V and OD1 demonstrated much larger shifts (C).

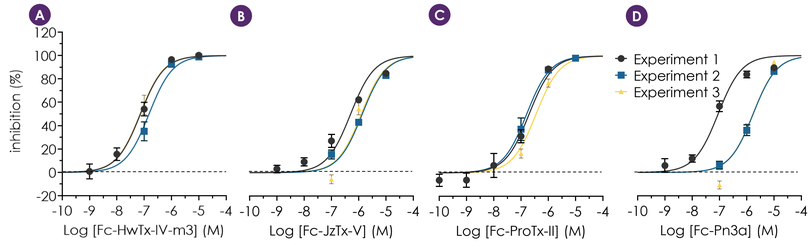


C

Toxin	Potency shift (fold-change)
HwTx-IV	296
HwTx-IV-m3	32
ProTx-II	82
JzTx-V	870
ODI	3475

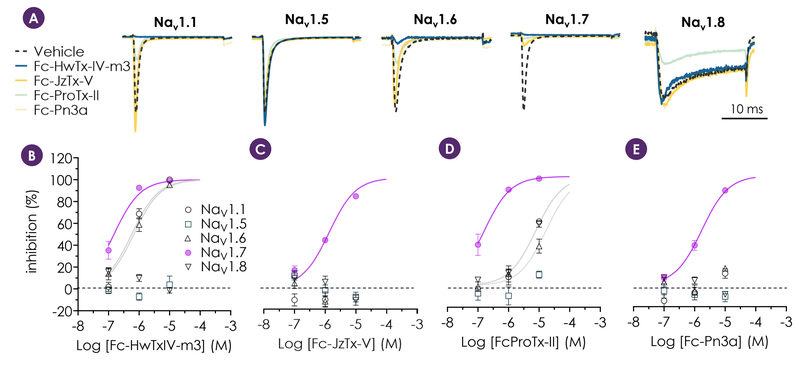
3. Reproducibility of Fc-toxin peptide fusions against $Na_V1.7$

Fc-toxin peptide reproducibility was examined across the experiments performed against $Na_V1.7$ for Fc-toxin fusions (A – D). HwTx-IV-m3, JzTx-V and ProTx-II all demonstrated good reproducibility.



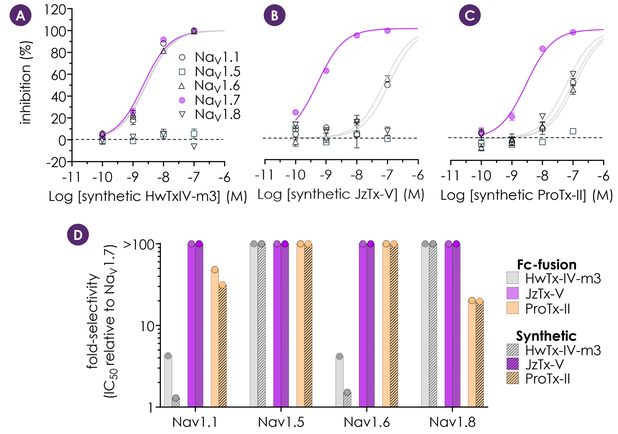
4. Selectivity profiling of Fc-toxin peptide fusions

Fc-toxin peptide fusions were counter-screened against cardiac sodium channel ($Na_V1.5$) and a range of sensory neuronal channels ($Na_V1.1$, 1.6 and 1.8) (A). Fc-HwTx-IV-m3 displayed acceptable potency (<1 µM) but a narrow selectivity window (<10-fold) (B). Both, Fc-JzTx-V and Fc-Pn3a displayed low potency (>1 µM) and good selectivity (>10-fold) (C, E), whereas Fc-ProTx-II demonstrated acceptable potency and selectivity (D).



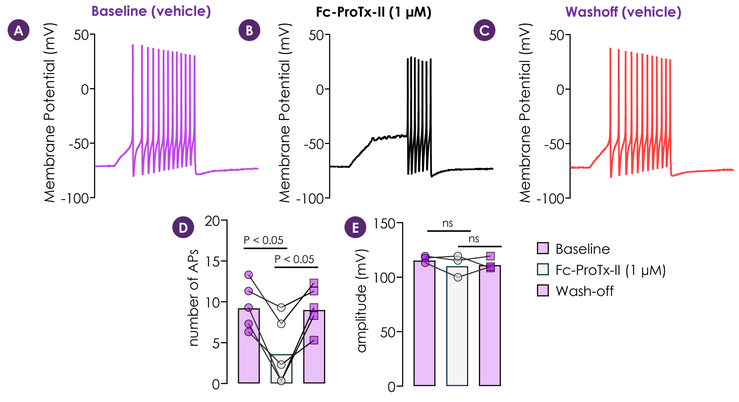
5. Selectivity comparison between synthetic and Fc-toxin peptide fusions

Selectivity profiles of three (commercially available) synthetic peptides were determined (A – C) and compared to the recombinant form. All Fc-toxin fusions demonstrated similar selectivity profiles (normalised to Nav1.7) to their synthetic counterpart, supporting the effectiveness of this strategy in producing viable peptides (D).



6. Effect of Fc-ProTx-II on neuronal function

Fc-ProTx-II was selected for neuronal excitability studies due to high potency, selectivity and reproducibility. Fc-ProTx-II reduced action potential firing in rat dorsal root ganglion neurons, which was reversible upon washout (A – C). The number of action potentials occurring upon Fc-ProTx-II application was significantly reduced (D), whereas action potential amplitude, which is governed by other Na_V channels, was unaffected (E), suggesting selective inhibition of $Na_V1.7$.



Conclusions

- Functional cysteine-rich toxin peptides can be produced using traditional protein purification methods via an Fc-fusion approach
- This approach could advance peptide research, accelerate SAR studies, and provide new therapeutic strategies

References

1. Sermadiras I *et al.* PLoS One. 2013 Dec 6;8(12):e83202
2. Smith JJ *et al.* J Biol Chem. 2007 Apr 27;282(17):12687-97
3. Gonzalez-Prada JE *et al.* (2026), manuscript submitted