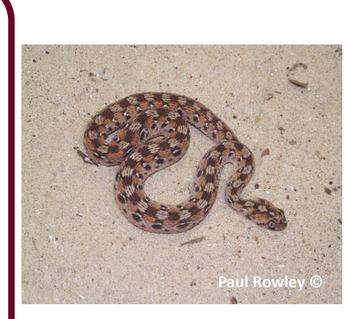
ADDovenom: Novel Snakebite Therapy Platform of Unparalleled Efficacy, Safety and Affordability

Stefanie K. Menzies[1,2], Rebecca J. Edge[1], Nicholas R. Casewell[1,2], Robert A. Harrison[1,2], Imre Berger[3], Christiane Schaffitzel[3]

[1]Centre for Snakebite Research & Interventions, Liverpool School of Tropical Medicine, Liverpool UK
[2] Centre for Drugs and Diagnostics, Liverpool School of Tropical Medicine, Liverpool UK
[3] BrisSynBio Bristol Synthetic Biology Research Centre, University of Bristol, Bristol UK

Background: Antivenom is the only effective treatment for snakebite envenoming yet suffers from numerous deficiencies, including poor toxin-specificity, low dose-efficacy, high incidence of adverse reactions and high cost to the patient. Antivenom is manufactured by injecting animals (horses/sheep) with sublethal doses of crude venom and the animals IgG is purified, thus this approach is suboptimal because it (i) does not take into account immunogenicity or toxicity of the venom proteins in crude venom, and (ii) only ~10% of the purified IgG are toxin-specific.

In this project, we aim to rationally design adenovirus-inspired toxin binding proteins (ADDovenom) to bind and neutralise the most medically-relevant toxins in the venoms of *Echis* and *Dendroaspis* species from sub-Saharan Africa

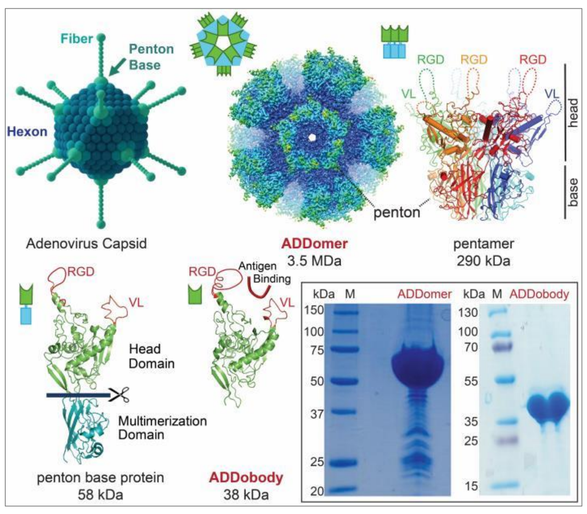


ADDovenom design and features

ADDobody protein is based on the head domain of adenovirus penton-base protein, which contains several loops that are flexible in sequence and length.

We will randomise the loops to generate a naïve ADDobody library with >10^{12} members and screen for high-affinity binders to different toxin targets.

The 38 kDa ADDobodies can multimerise to form 60-mers termed 'ADDomers', resulting in 60 binding sites on the 3.5 MDa multimer.



Advantages over antivenom:

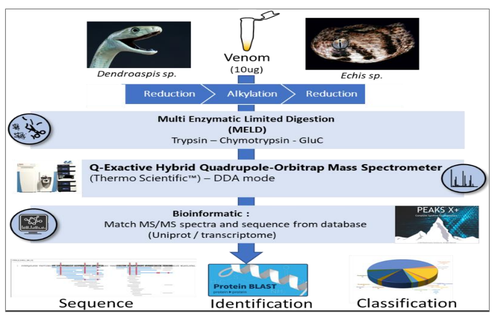
- 38 kDa ADDobodies are suited to topical application and transcutaneous delivery to neutralise necrosis-causing toxins
- High avidity ADDomers with 60 binding sites suited for neutralising systemic toxins
- Heat stable (does not require cold chain)
- Can be lyophilised to extend expiry dates
- Can be produced in insect and bacterial cells with exceptionally good yields
- The chimpanzee adenovirus penton base protein is immune neutral in humans

Planned work

Mass Spectrometry and Bioinformatics

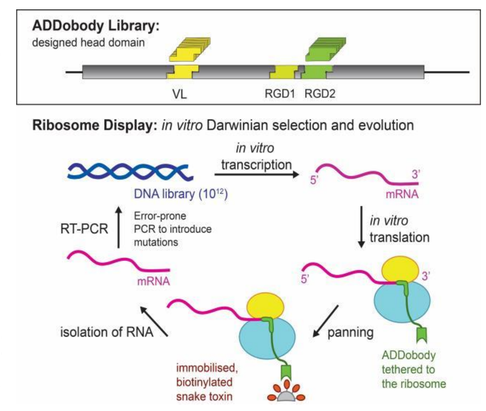
We will use LC-MS/MS to characterise the crude venom from 4 *Echis* species and 5 *Dendroaspis* species. We will define toxin mass, sequence, and abundance.

From this we will generate a toxin database, identify common sequences, and then identify and select the most potent toxins to screen the ADDobody library against. In parallel we will use bioinformatics to design consensus toxins and identify highly-conserved toxin epitopes to be used as targets.



In vitro evolution and characterisation of ADDobody binders

We will express and purify toxins identified from the MS data, then generate the naïve ADDobody library, confirm loop diversity, and adapt this for ribosome display. We will then select ADDobodies with the strongest toxin binding and perform *in vitro* evolution to maximise affinity.
Selected ADDobodies will be scaled up, characterised by SPR and thermal shift assays, and multimerised to create ADDomers.



Examination of neutralising ability

In vitro

We will test ADDobodies and ADDomers for *in vitro* neutralisation of toxin activities using toxin-specific and phenotypic assays, including:

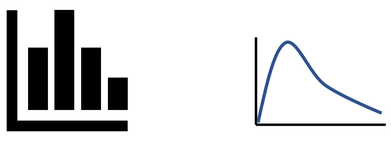
- Disturbances to plasma clotting
- Phospholipase A2 activity
- Cell cytotoxicity
- Snake venom metalloproteinase activity
- Inhibition of nicotinic acetylcholine receptors
- Inhibition of Kv1.1 channels

We will also use serological assays (ELISA and Western) to assess binding

In vivo

Top candidates identified through *in vitro* studies will progress to *in vivo* testing in murine models, to determine:

- ED50 against venom-induced lethality for each venom, and compared to existing antivenoms
- eMND (dose required to prevent necrotic lesions) for *Echis* venoms
- Safety and toxicity
- Pharmacokinetics

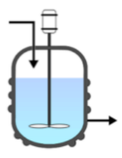


Scalable Bioprocess for ADDomer Production

We will establish a GMP-compatible platform for production of ADDovenom, by:

Production and purification of ADDovenom

- Baseline vs optimized process using insect cells and small scale bioreactors (up to 2 L)
- Documentation for easy transfer to pilot- and full-scale production level



Up- and down-stream process development

- Define critical process parameters
- Supplement screening assisted by Design of Experiments
- Scaled-down models for downstream processing optimization
- High cell density cultures in small-scale bioreactors

Implement analytical methods

- SDS-PAGE
- Western blot
- ELISA
- Transmission electron microscopy
- Thermal shift assay
- Nanoparticle tracking analysis
- Dynamic light scattering

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