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Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

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Design of Fab-based chimeric antibodies against *Bothrops asper* toxins

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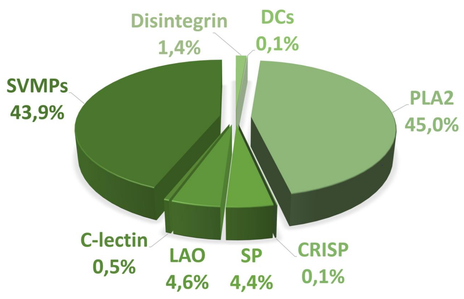
1 Addressing the problem of immunogenic antivenoms

Snakebite is one of the world's most neglected tropical diseases, with an estimated 5 million bites per year, resulting in about 125.000 deaths.[1] The only current treatment for snakebite envenoming is antiserum derived from the blood of immunized mammals (typically horses).[2] These antisera are expensive to produce and carry a high risk of causing hyper-allergic reactions in human recipients due to their heterologous origin.[3] Here we report the discovery of chimeric scFvs against *Bothrops asper* toxins.

2 Composition of *B. asper* venom



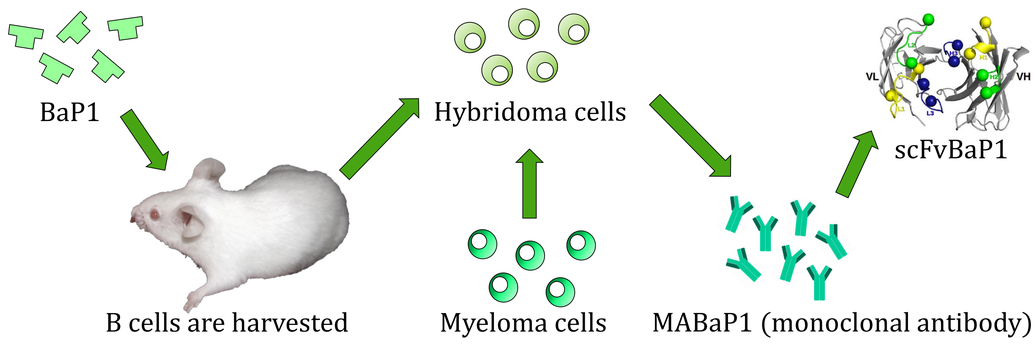
Composition of *B. asper* venom[4] according to protein families, as percentages of the total protein content:

SVMPs:	Snake Venom MetalloProteinase
PLA_2:	Phospholipases A_2
CRISP:	Cysteine-Rich Secretory Proteins
LAO:	L-amino Acid Oxidases
SP:	Serine Proteinases
DC:	Dendritic Cell Fragments

Results presented here were obtained prior to the project[4]

3 Construction of scFvBaP1 from a monoclonal antibody

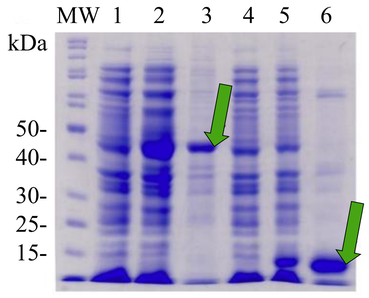
Hybridoma cells secreting the monoclonal antibody against BaP1 (MABaP1) were made, and from these a recombinant single chain variable fragment (scFv) was constructed.

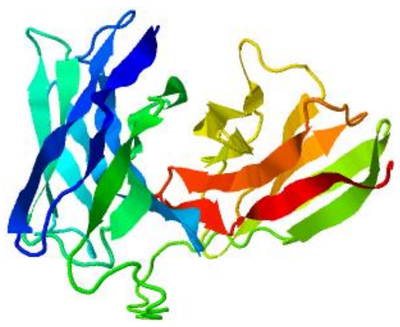


Results presented here were obtained prior to the project[5]

4 Results from the recombinant scFvBaP1

The scFvBaP1 was cloned into the pMST3 expression vector in frame with a SUMO (**S**mall **U**biquitin-like **Mo**difier) sequence. When subjected to SDS-PAGE, the sequenced SUMO-scFvBaP1 showed a band at 40 kDa as was predicted and SUMO protein around 13 kDa[5] (well 3 and 6 respectively).



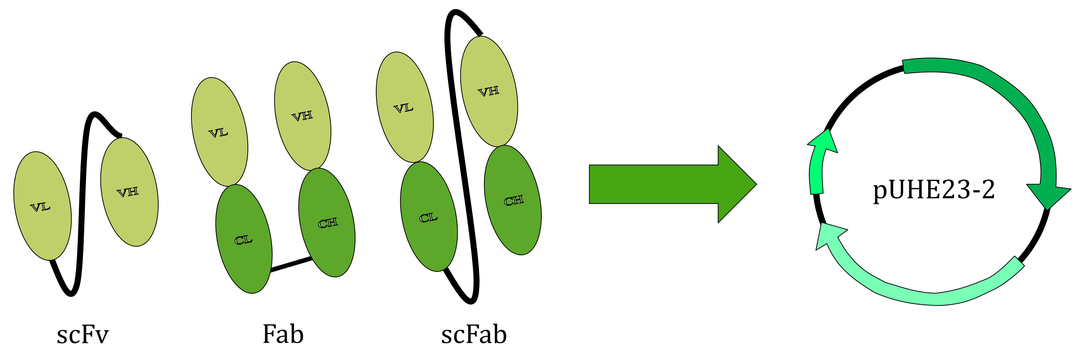


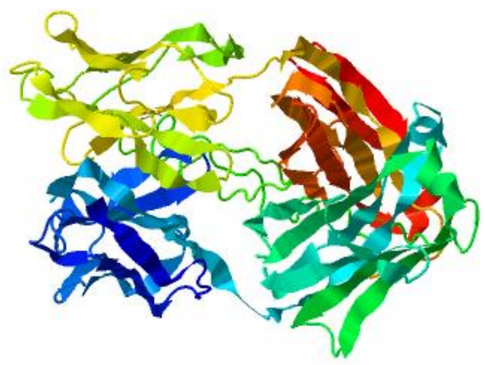
In vivo tests showed that scFvBaP1 specifically recognizes BaP1 and whole *B. asper* venom, and to a large extend neutralizes the main toxic effects of BaP1.

Results presented here were obtained prior to the project[5]

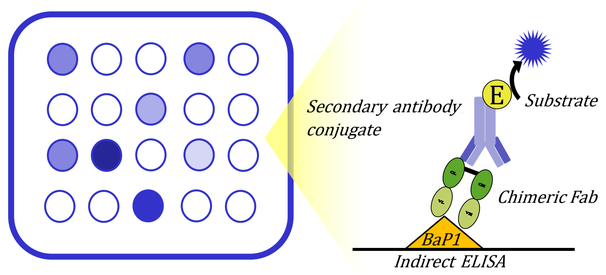
5 Transformation of antibody formats into *E. coli*

Recombinant FabBaP1 and scFabBaP1 will be made from scFvBaP1. These are going to be expressed in *Escherichia coli* using a pUHE23-2 expression vector. The final protein model shown here is of the recombinant scFab.[6]





6 Binding strength testing of chimeric antibodies by ELISA



Binding strengths of the antibodies to BaP1 will be determined by ELISA. This will allow us to compare the different antibody formats in regards to how likely they are to neutralize BaP1.

7 Next steps: Determining the best antibody format

If good chimeric antibodies are obtained in good yield, the next steps will be to determine their K_D and test the antibodies in a preclinical model. This will allow an *in vivo* comparison between the different formats.

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Acknowledgements

We thank the Novo Nordisk Foundation (NNF16OC0019248) and Symphogen A/S for financial support.