



Antibody Engineering & Therapeutics Europe

Recombinant antivenom based on oligoclonal broadly-neutralizing antibodies

Andreas Hougaard Laustsen-Kiel

Technical University of Denmark











Antibody Discovery

Antibody Discovery



Antibody Discovery

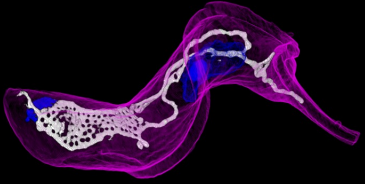
There it is!





Tropical Pharmacology Lab
Center for Antibody Technologies

Center for Antibody Technologies



Parasitic diseases

Scientific focus areas

Multi-protein targeting

Nanobody technology

Special properties (pH-sensitive)

Broadly-neutralizing antibodies

Affordable biotherapeutics for NTDs

Microbiome precision engineering

Bacterial diseases



Snakebite antivenoms



Industrial biotech & BioAg

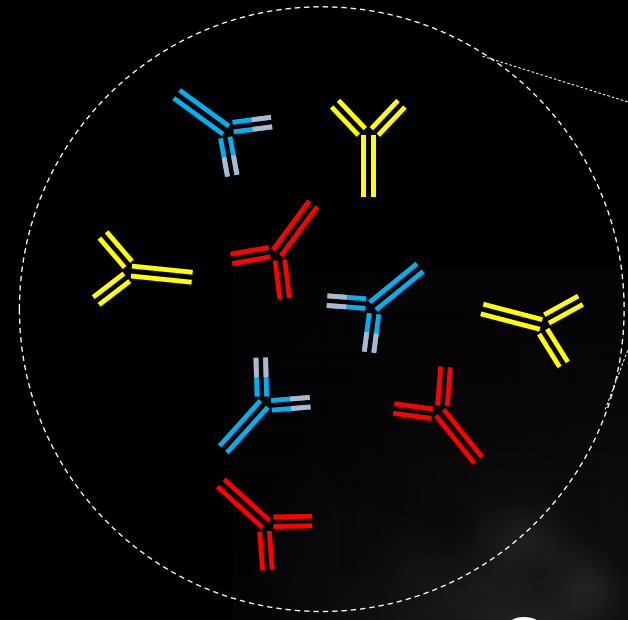
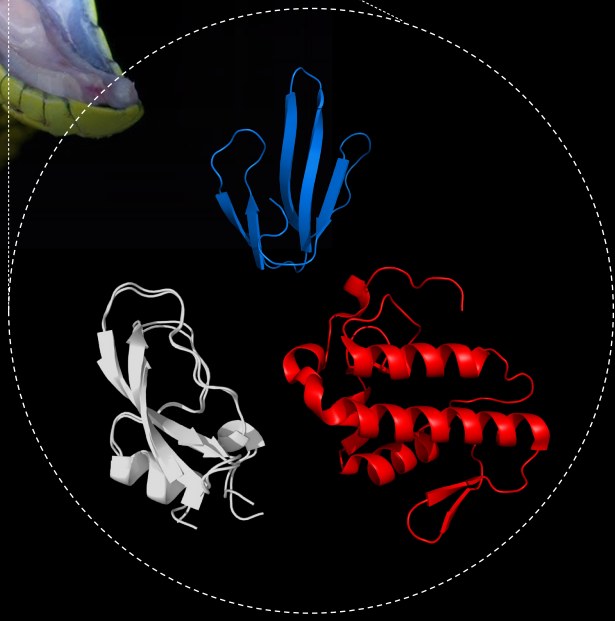




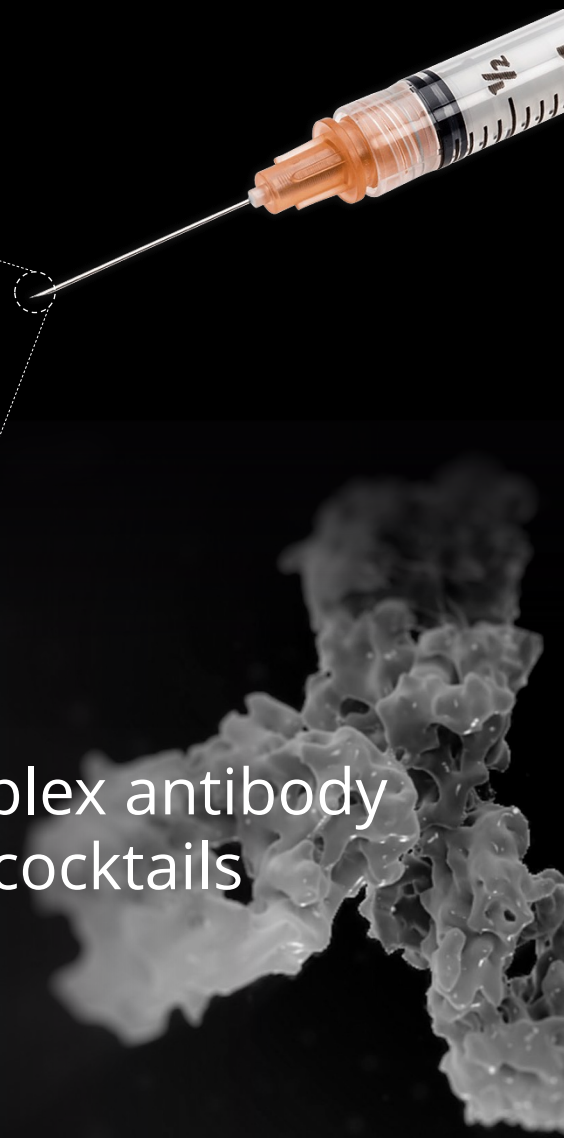
Recombinant oligoclonal broadly-neutralizing antivenom



Complex cocktails
of toxic proteins



Complex antibody
cocktails



Tanzania 2011

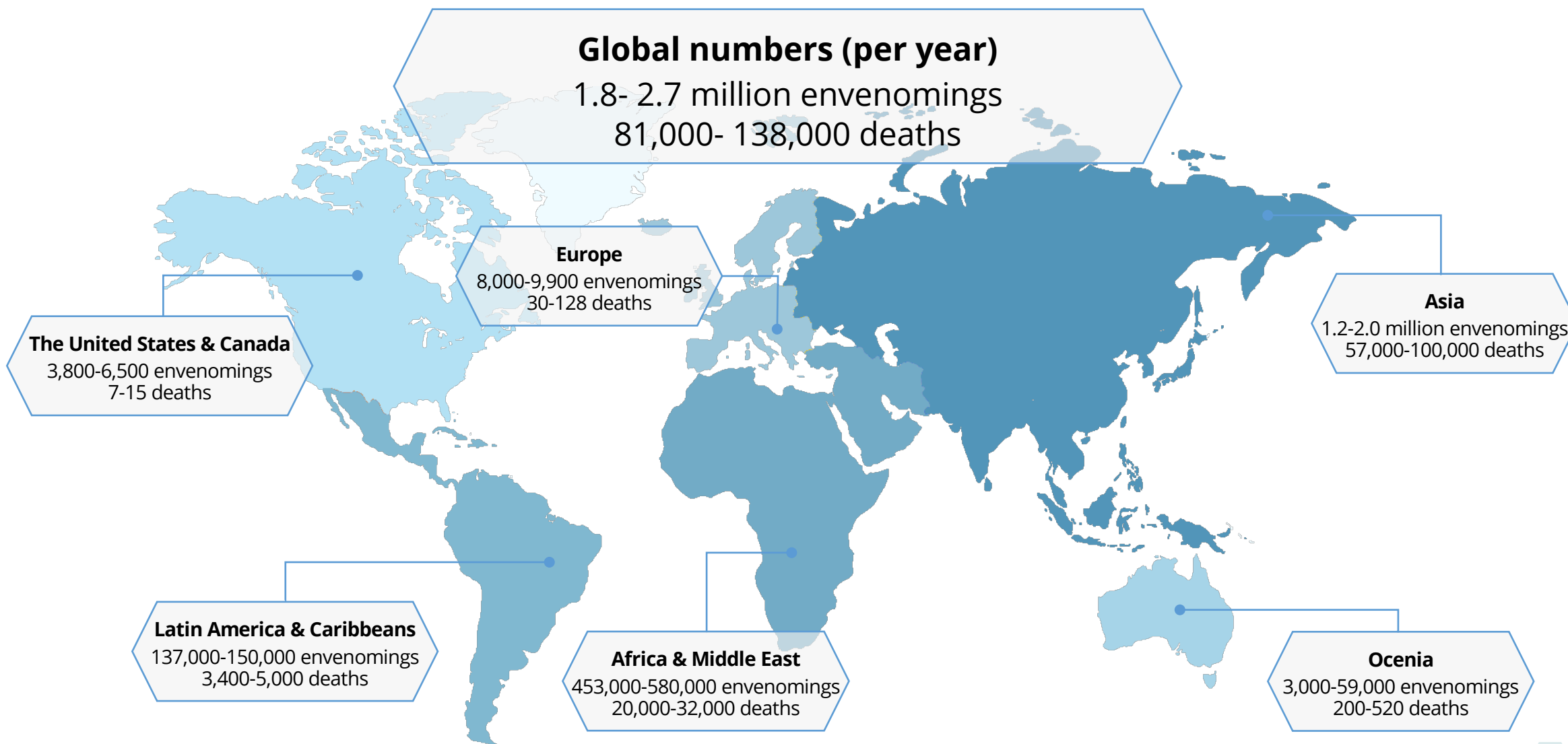




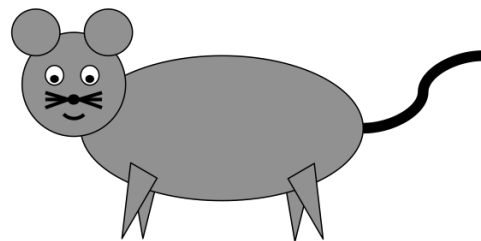
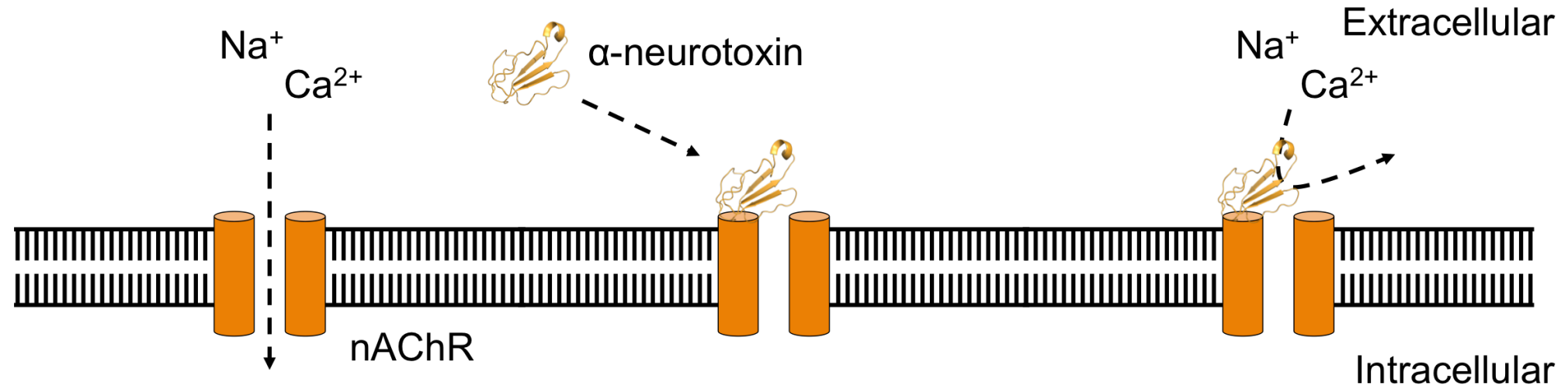




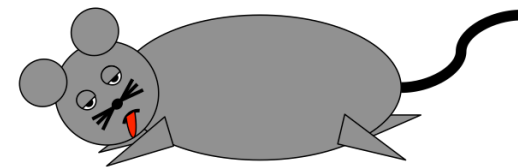
Snakebite envenoming claims millions of lives



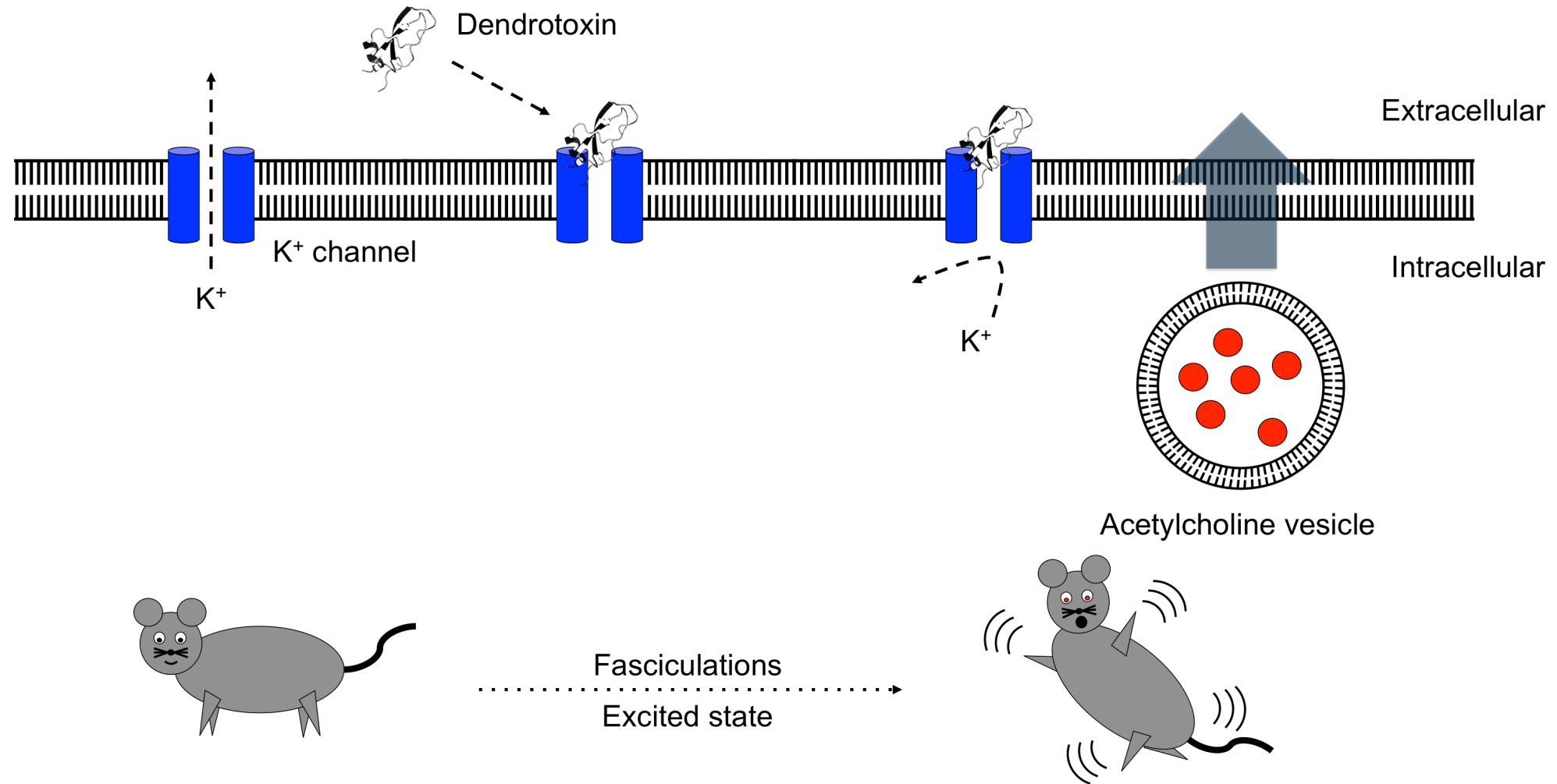
Neurotoxic snakebites – Effect of alpha-neurotoxins



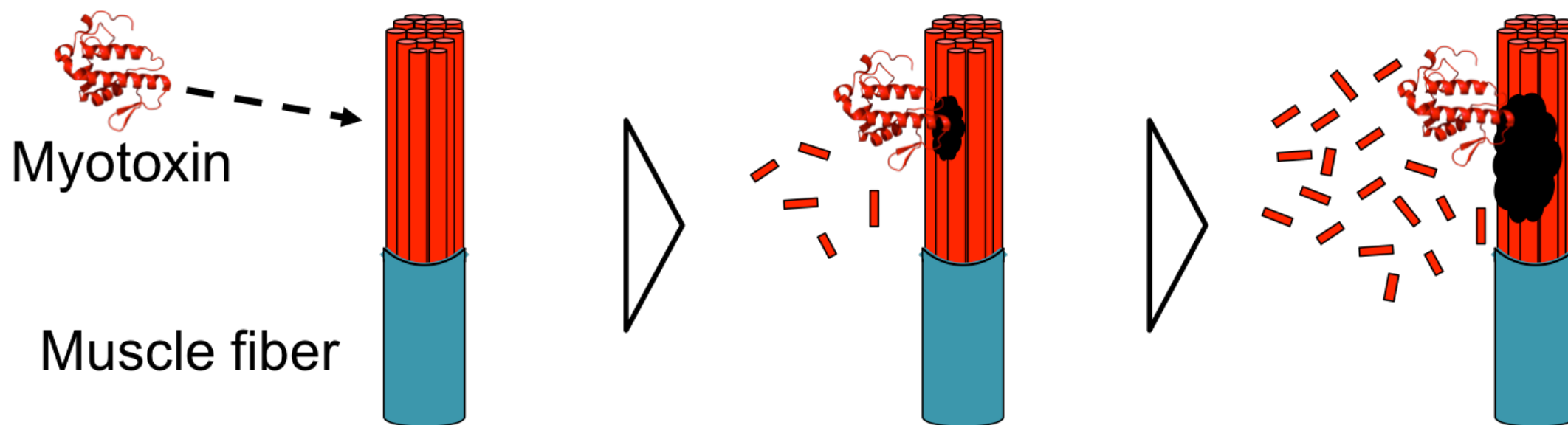
Flaccid paralysis
Respiratory failure



Neurotoxic snakebites – Effect of dendrotoxins



Myotoxic snakebites – Effect of myotoxins



Are snakes venomous or poisonous?

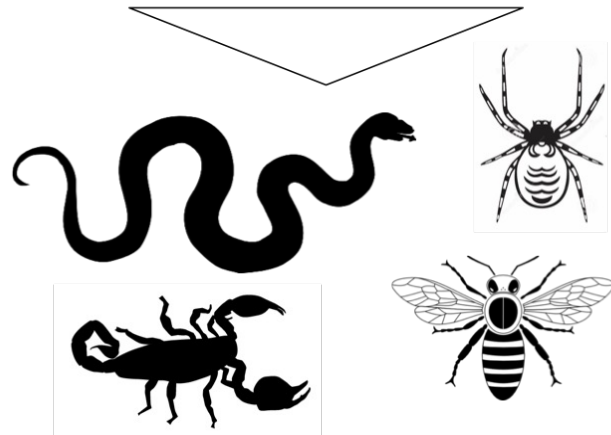
VENOMS



Toxic when injected



Not toxic when ingested



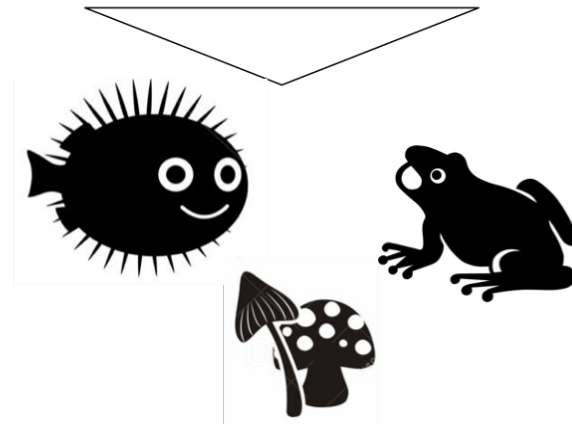
POISONS



Toxic when injected

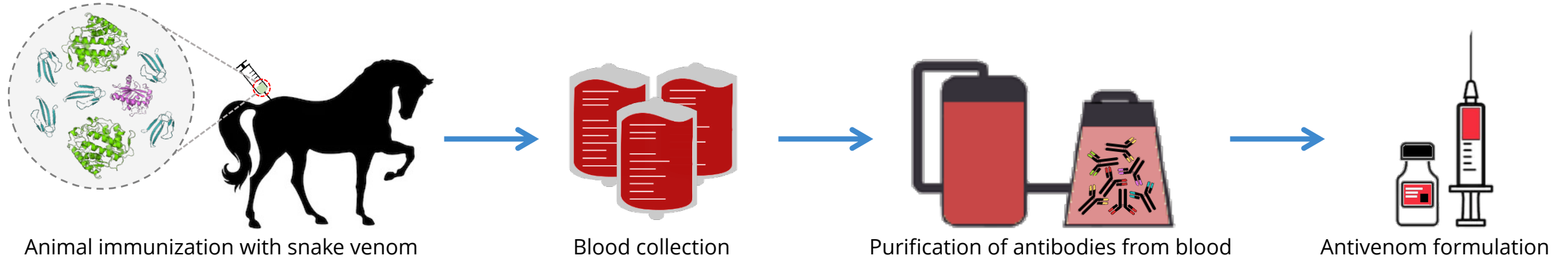


Toxic when ingested

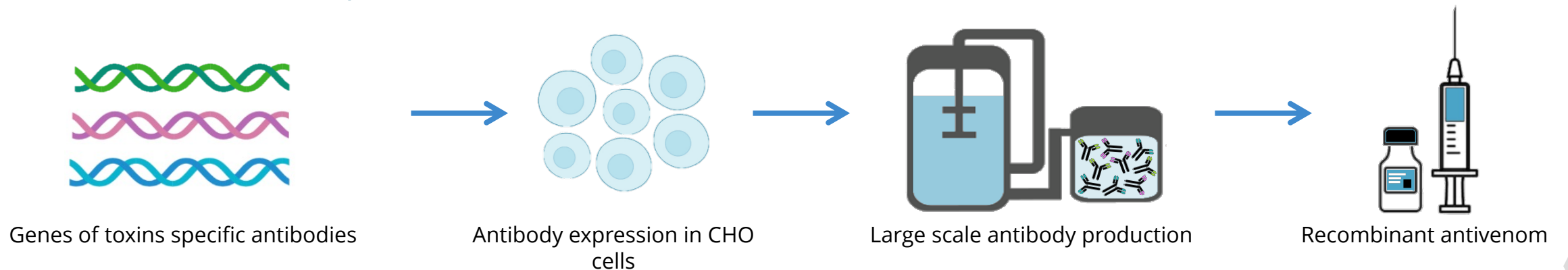


Antivenoms: a century old invention

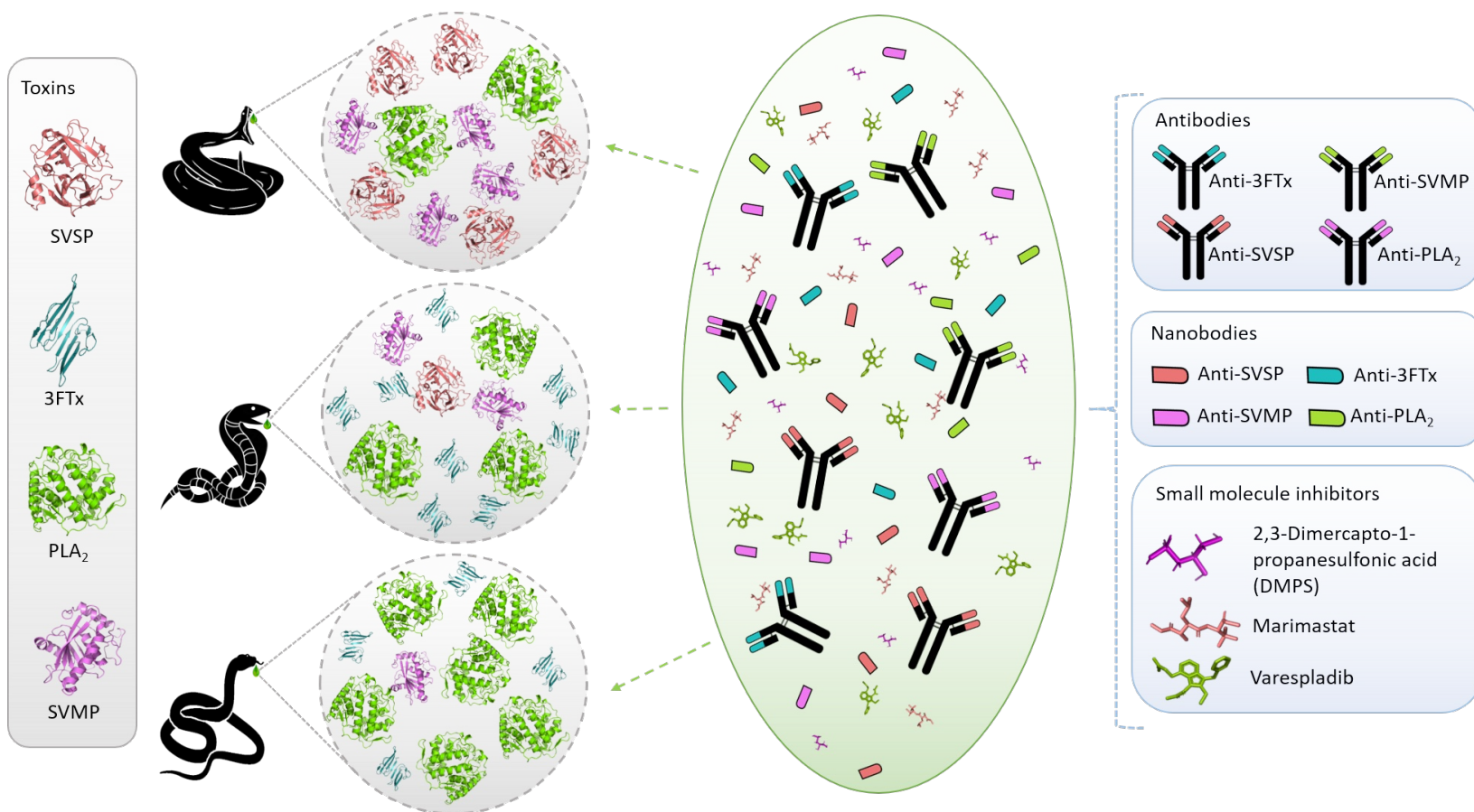
Traditional antivenom production



Recombinant antivenom production



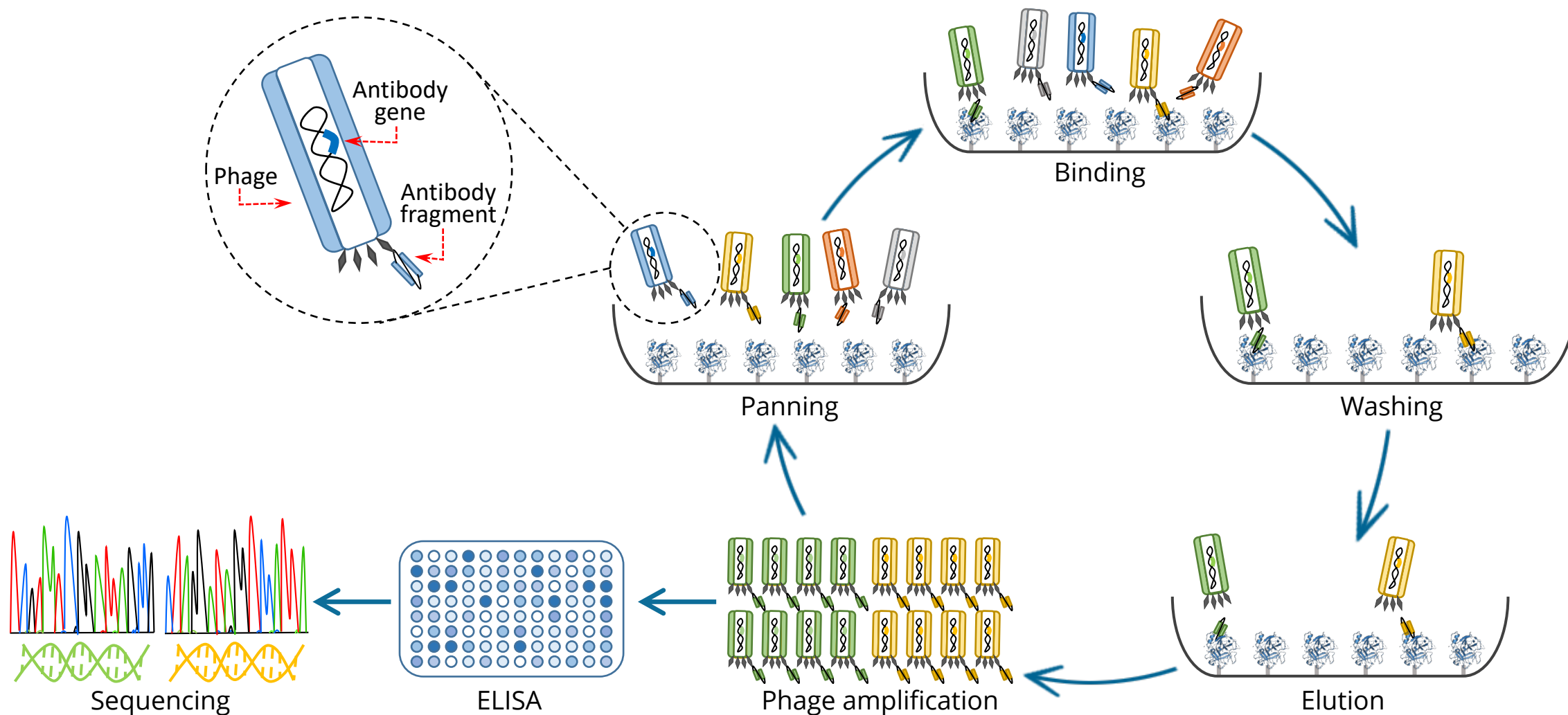
Recombinant antivenoms based on monoclonal antibodies



Benefits

- Compatibility with human victims
- Enriched for toxin-neutralizing antibodies
- Consistent and reproducible production
- Tailor-made antibodies with optimal pharmacokinetics (PK) and pharmacodynamics (PD)
- Rapid administration of antivenoms
- Acceptance among clinicians
- No dependence on snakes and horses
- Potential prophylactic use

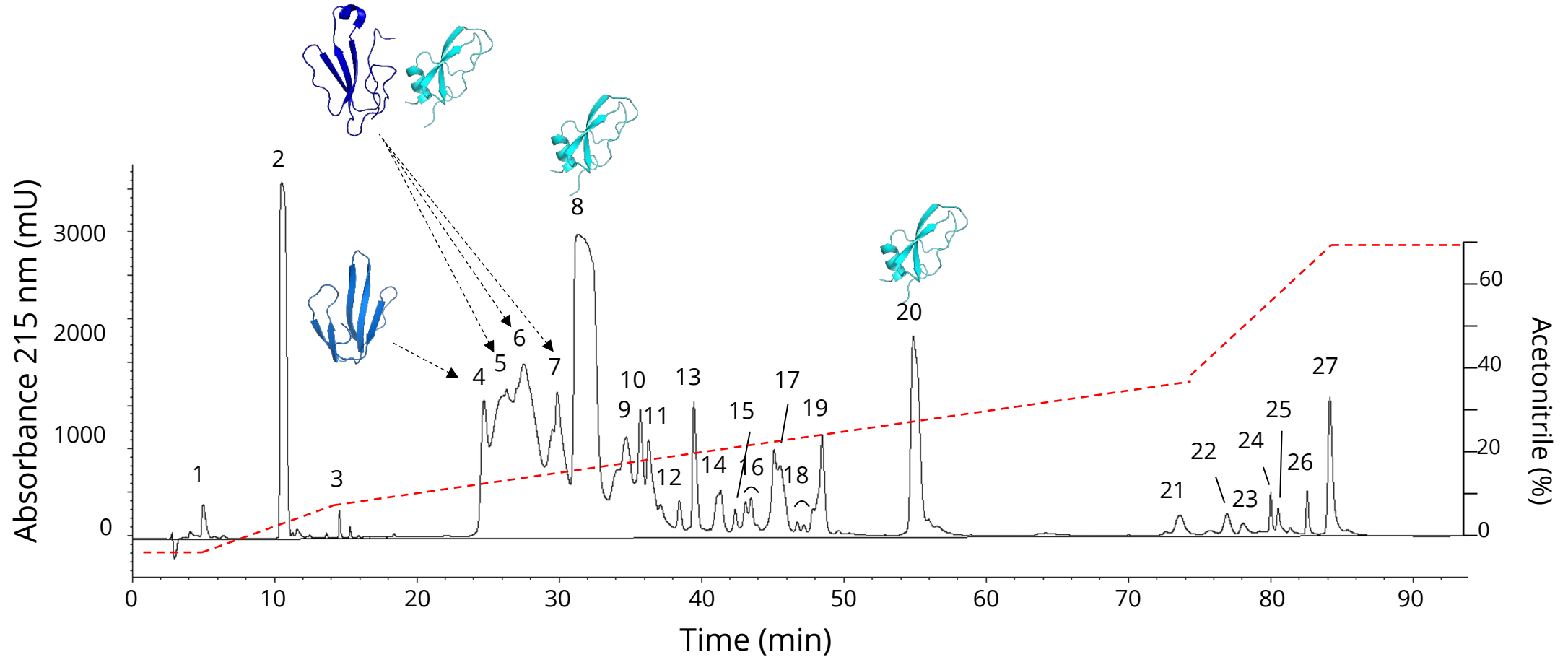
Phage display technology



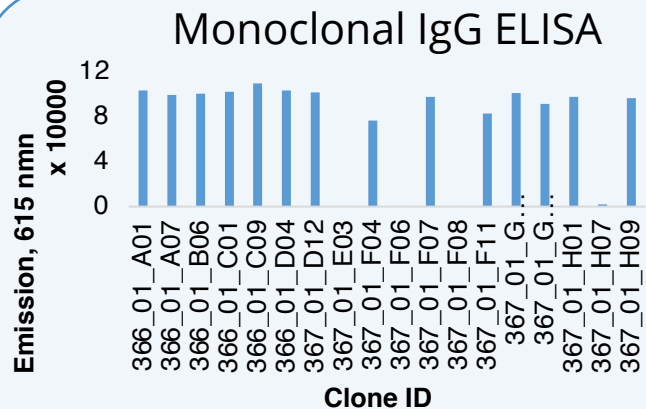
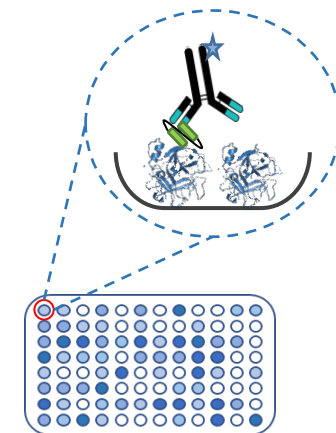
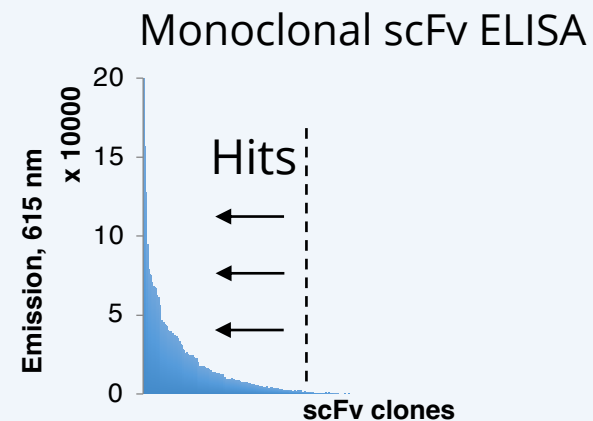
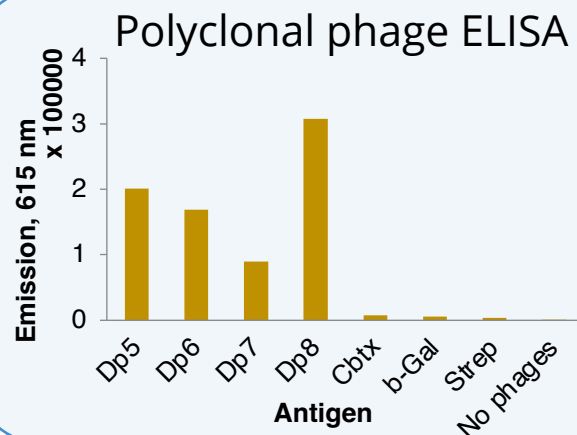
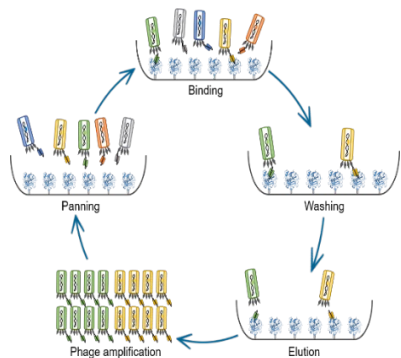




Oligoclones against black mamba venom

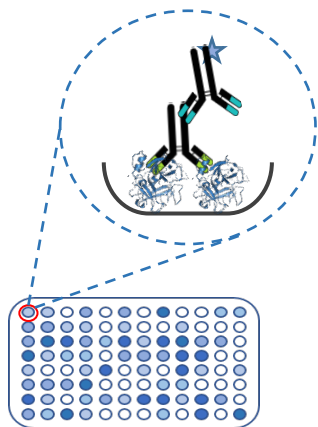
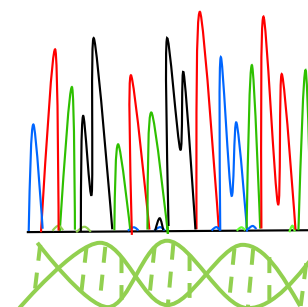


Oligoclonal antibodies against black mamba venom



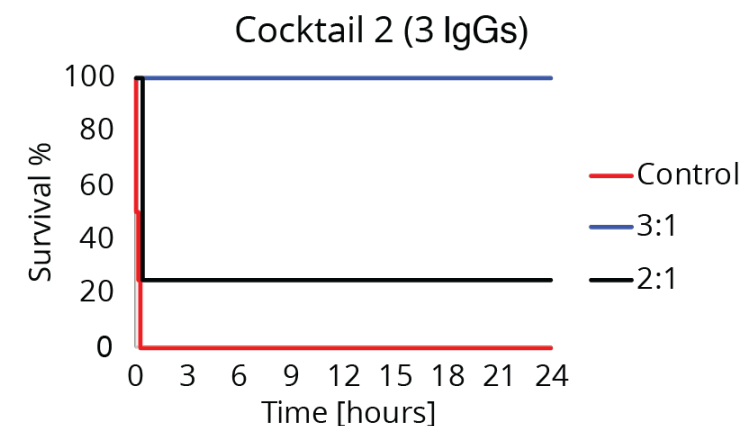
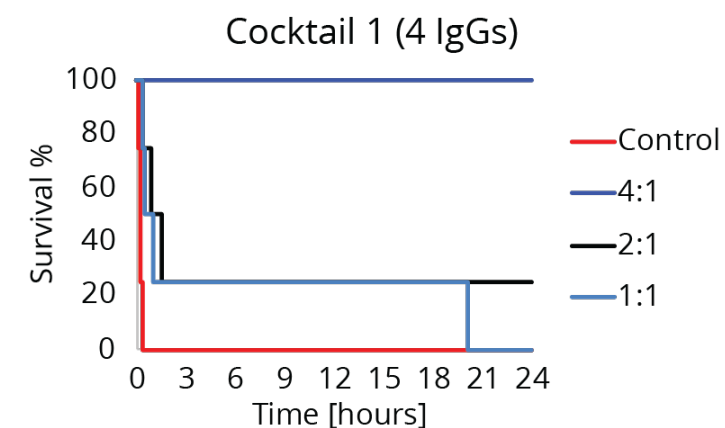
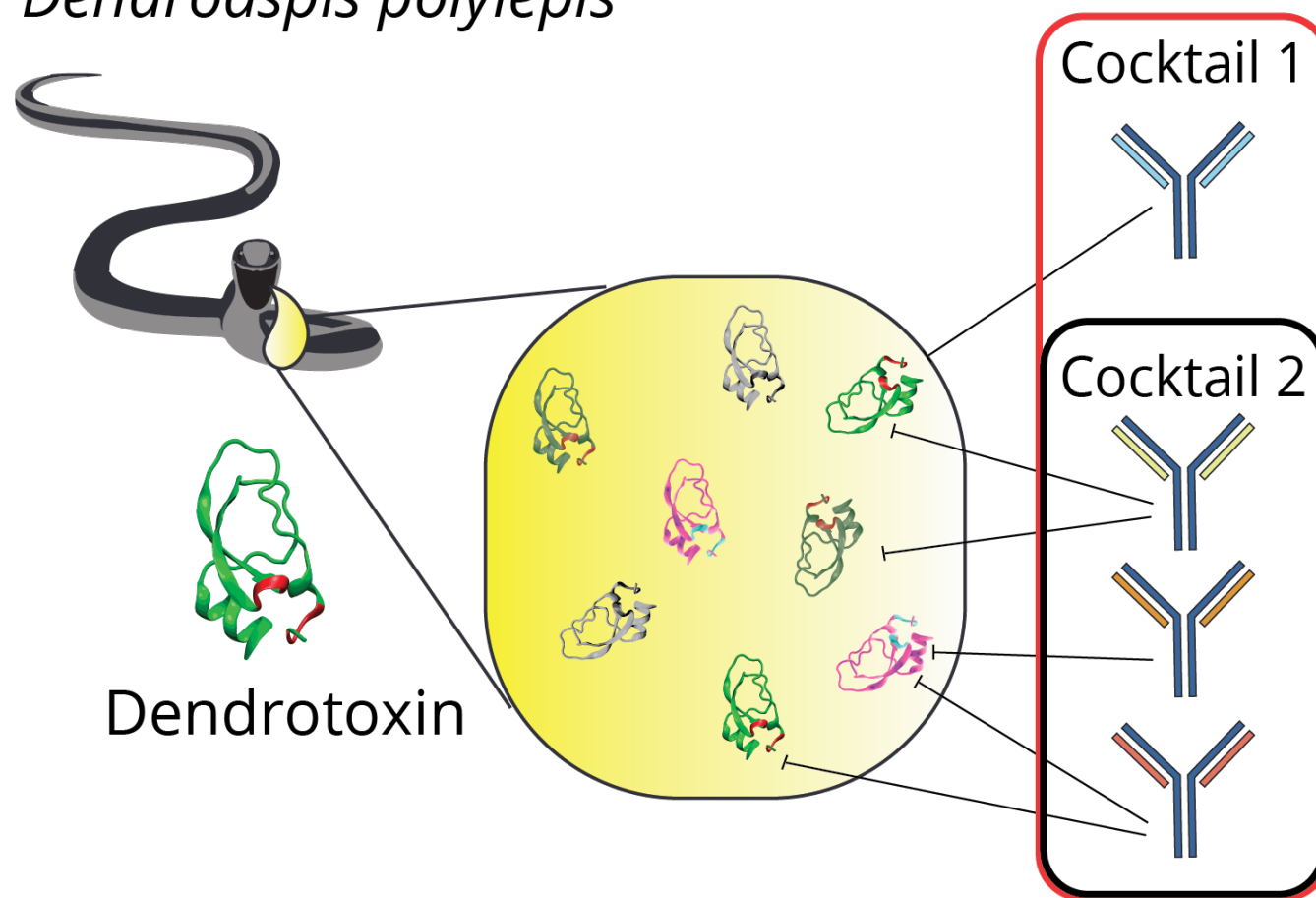
DNA sequencing

90 hits sequenced
35 unique V_H+V_L CDR3
29 unique V_H CDR3



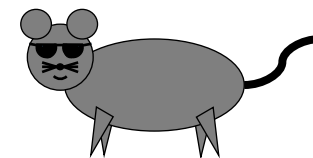
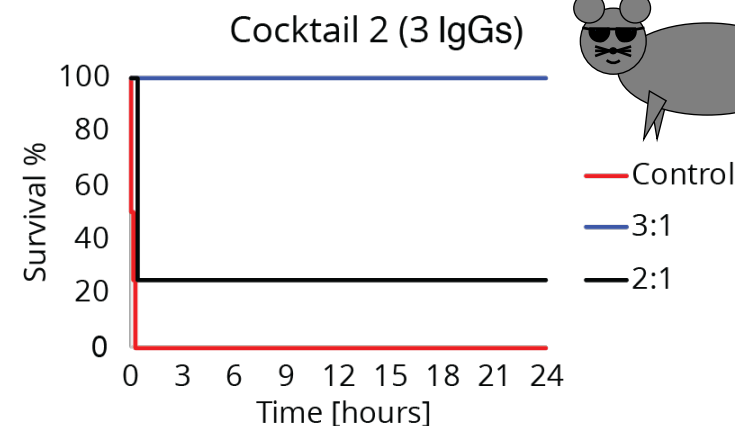
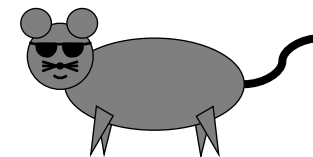
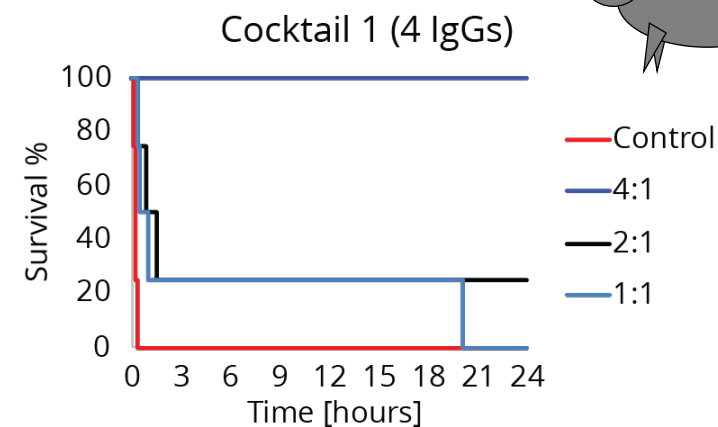
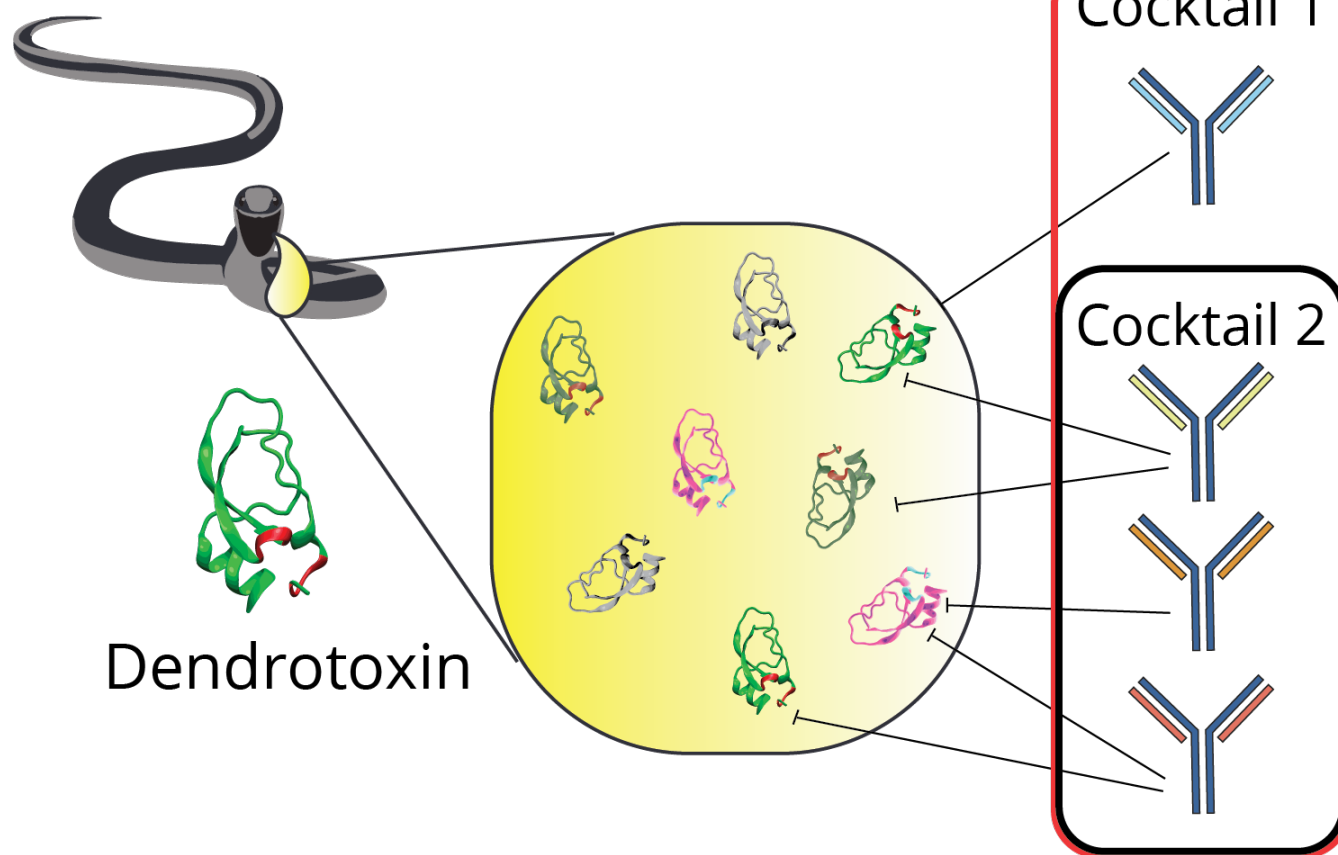
Oligoclonal antibodies against black mamba venom

Dendroaspis polylepis



Oligoclonal antibodies against black mamba venom

Dendroaspis polylepis







Wellcome: Recombinant antivenom for sub-Saharan Africa

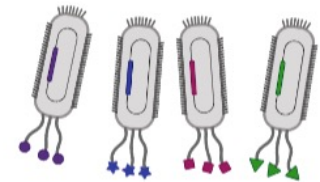


18 elapids

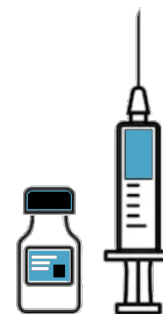
8 viperids



Immune
 V_H H-libraries



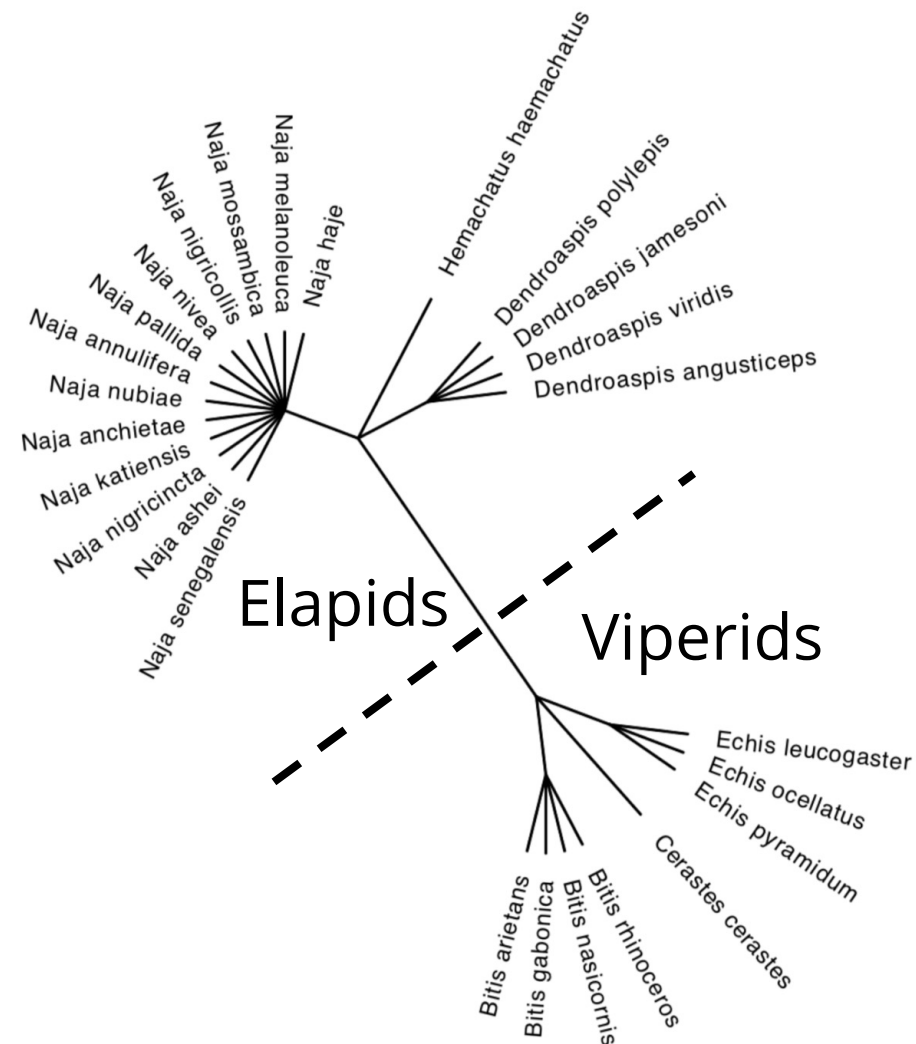
Naïve
scFv-library



Polyvalent
recombinant
antivenom

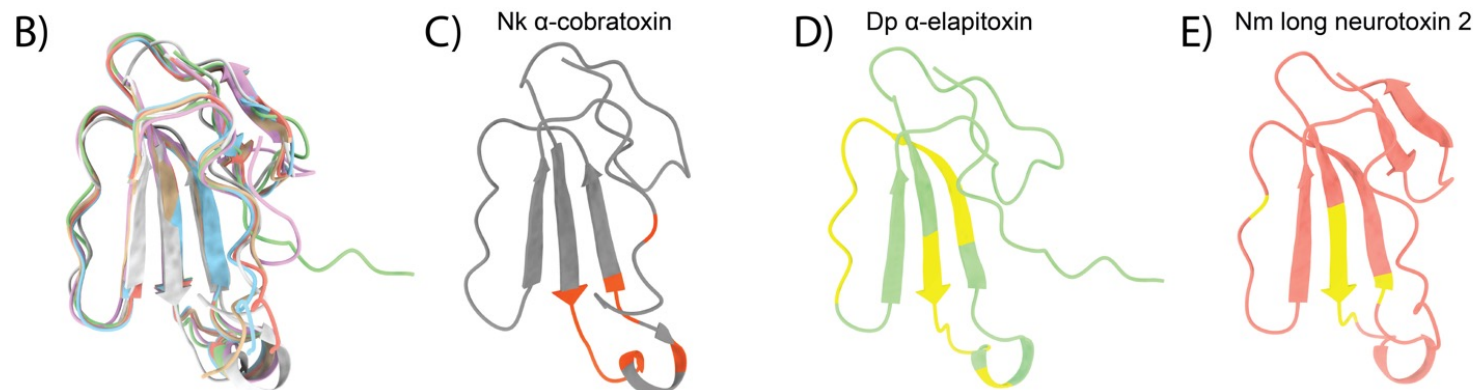
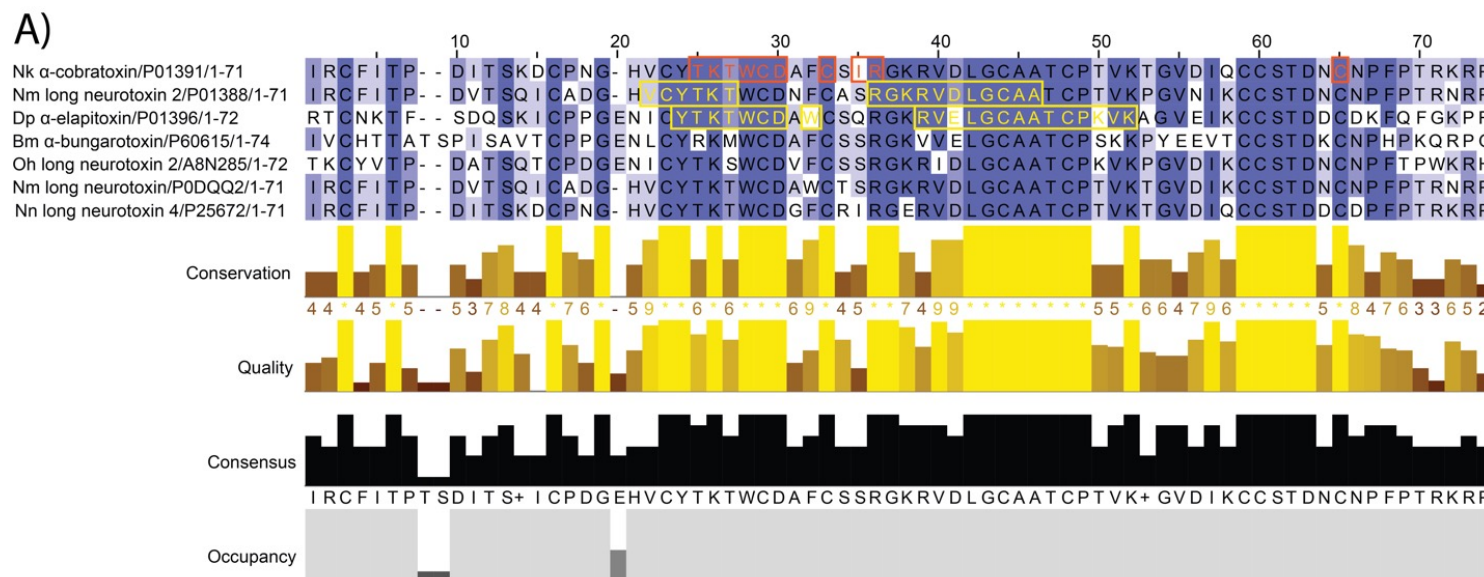


26 most medically
relevant snakes of
sub-Saharan Africa

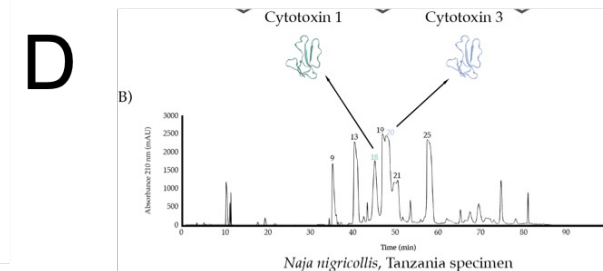
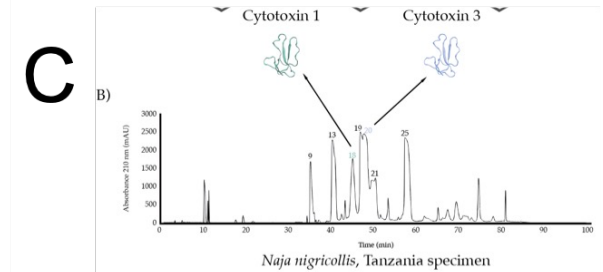
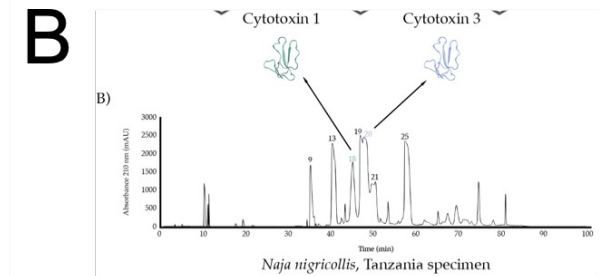
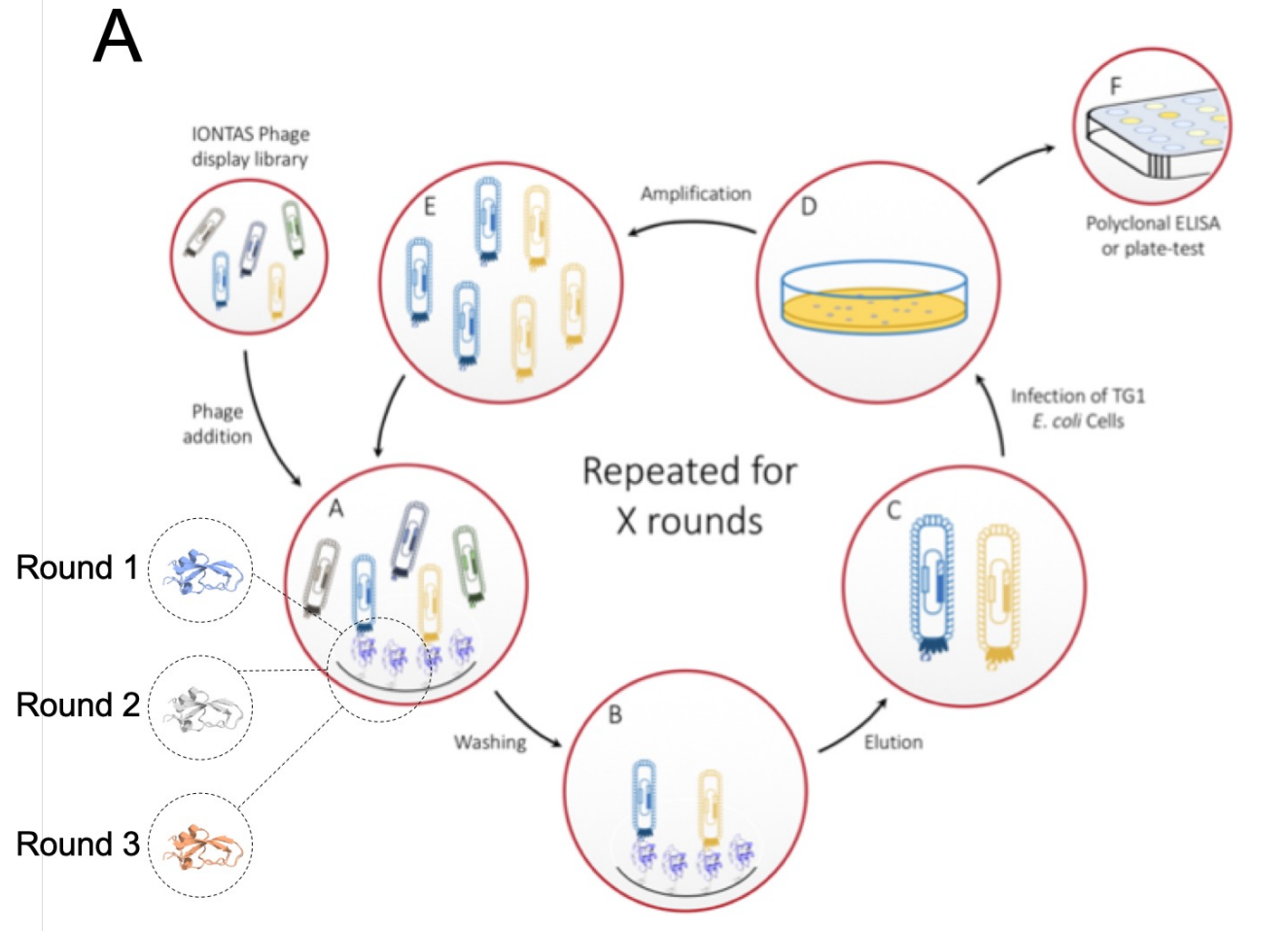




Broadly-neutralizing antibodies against α -neurotoxins

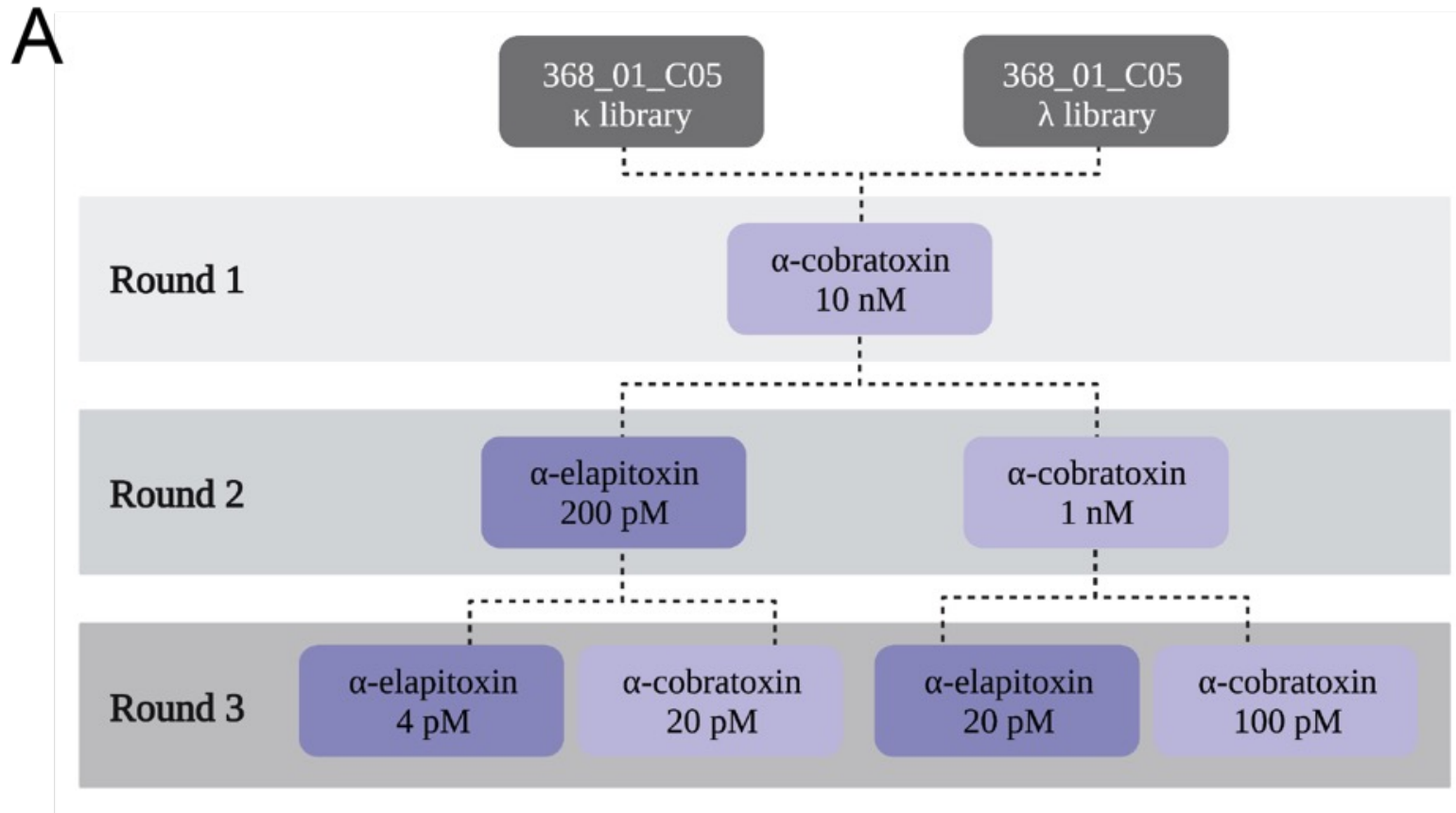


Cross-panning to increase antibody cross-reactivity



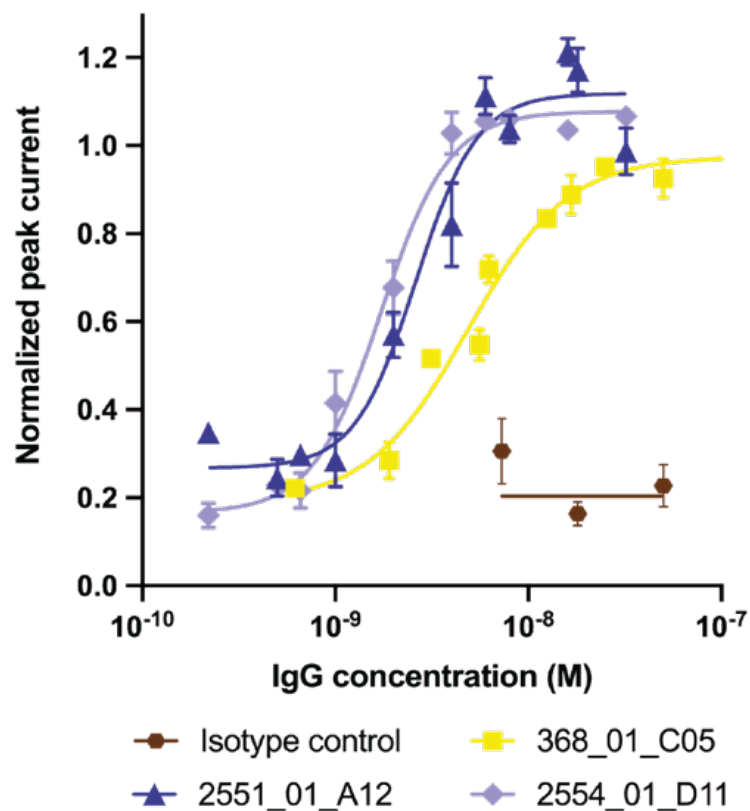


Broadly-neutralizing antibodies against α -neurotoxins

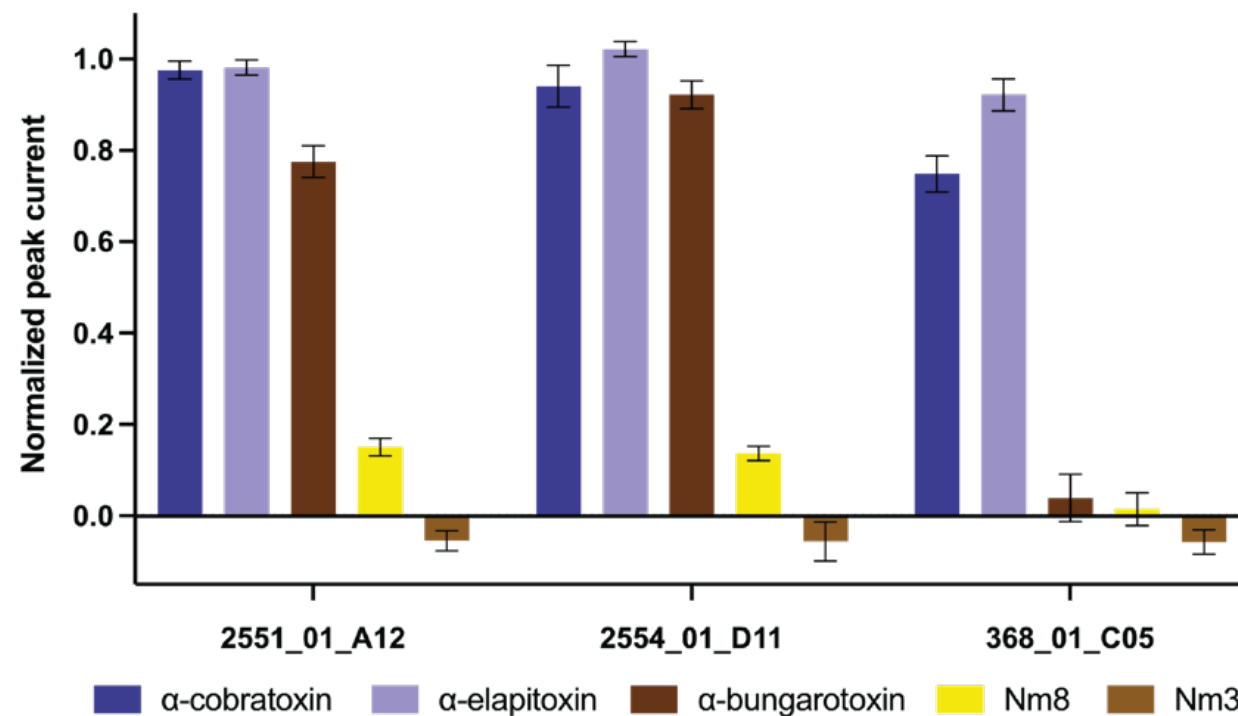


Broadly-neutralizing antibodies against α -neurotoxins

A)

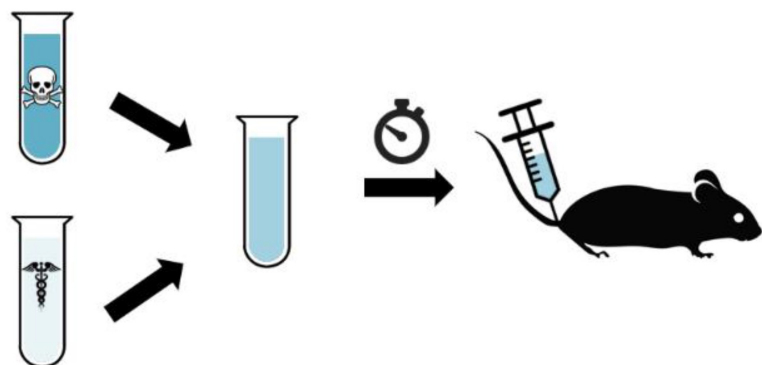


B)



Broadly-neutralizing antibodies against α -neurotoxins

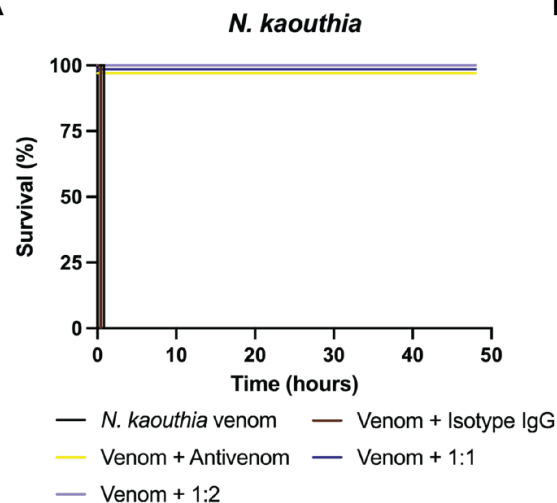
Preincubation assay



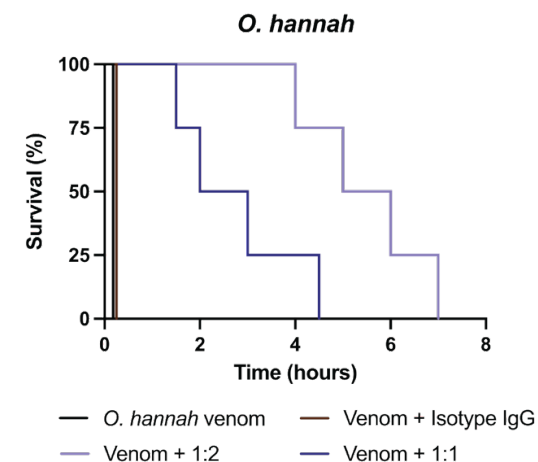
Rescue assay



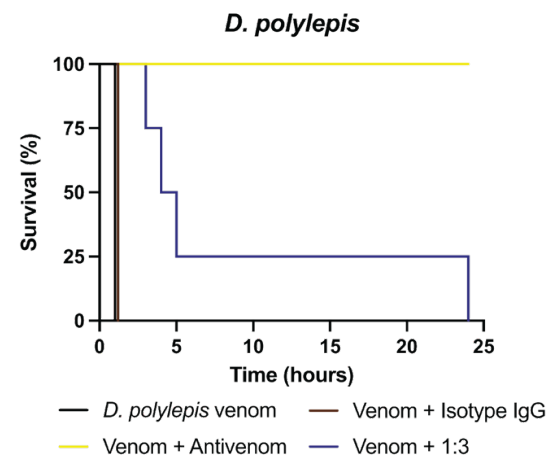
A



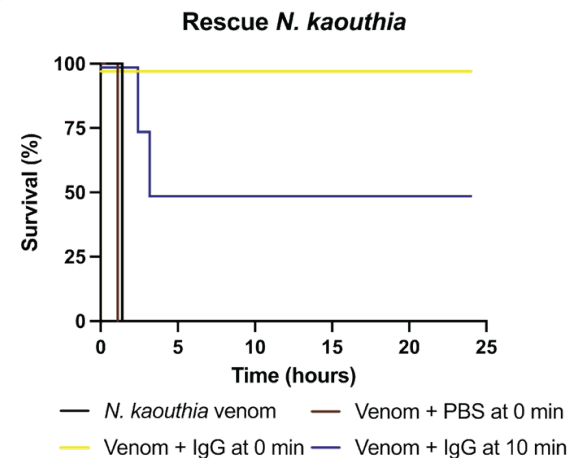
B



C

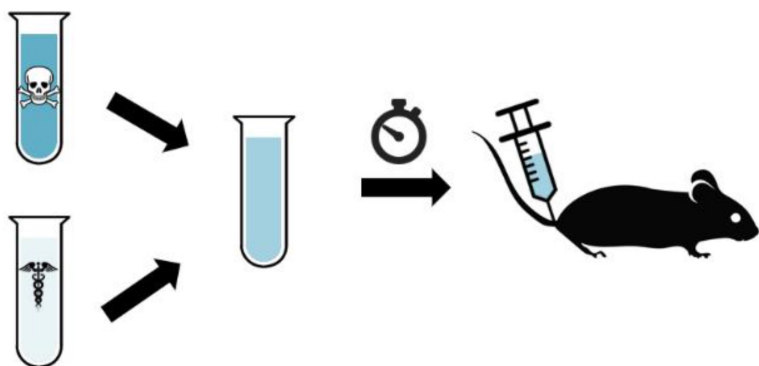


D



Broadly-neutralizing antibodies against α -neurotoxins

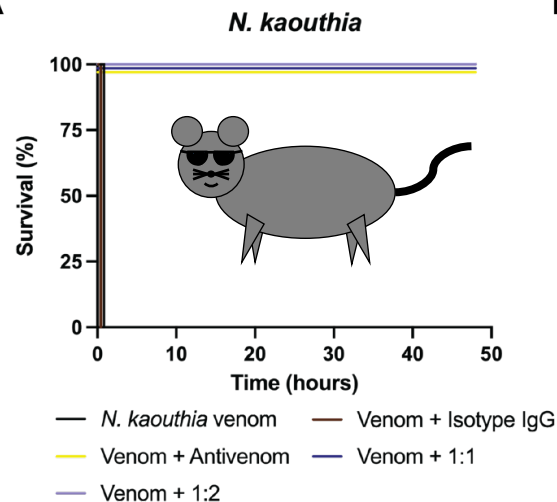
Preincubation assay



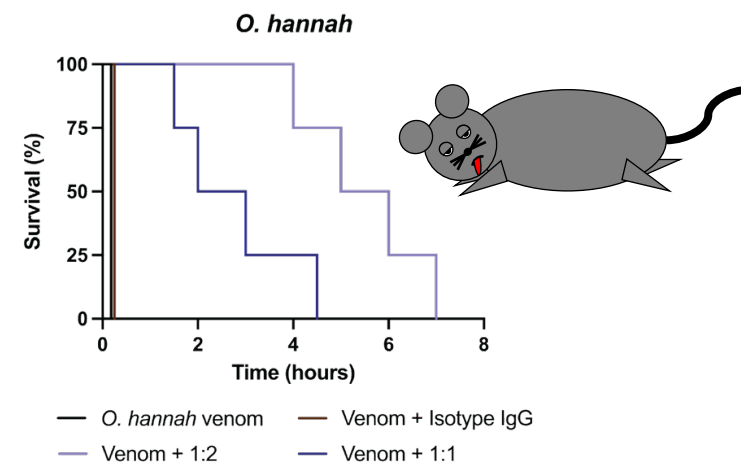
Rescue assay



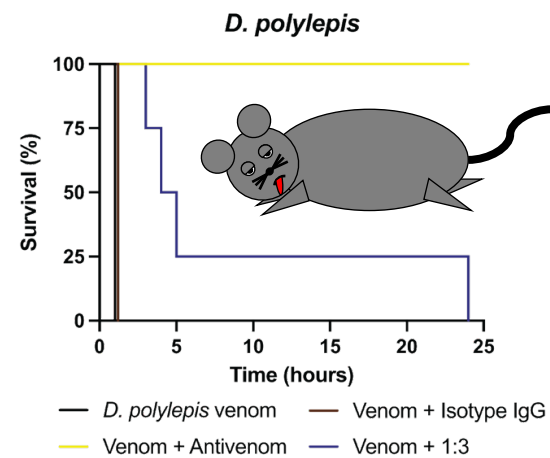
A



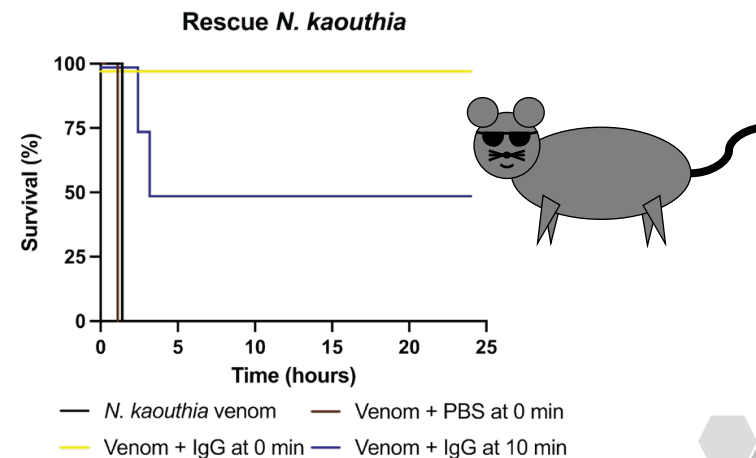
B



C

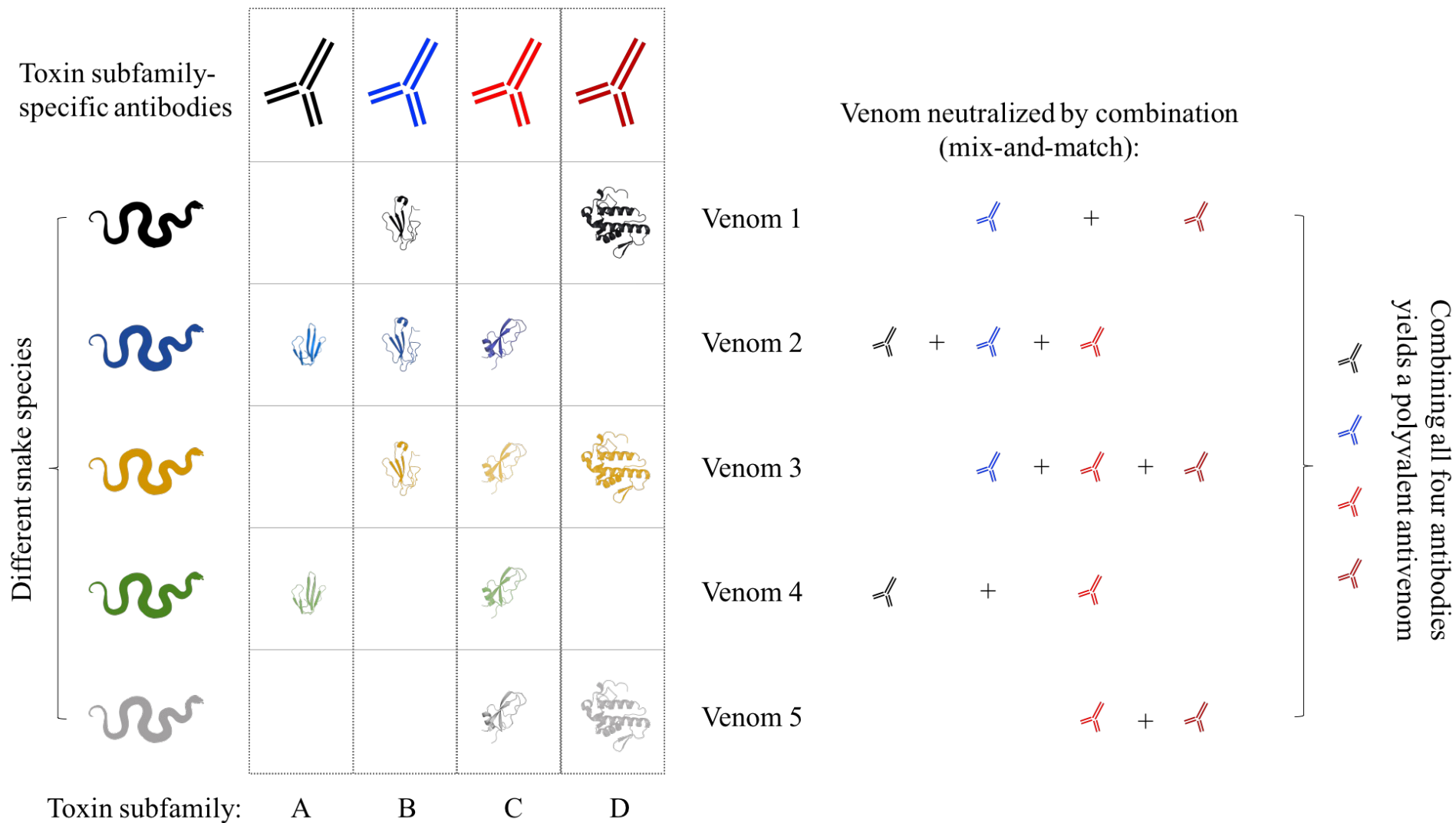


D





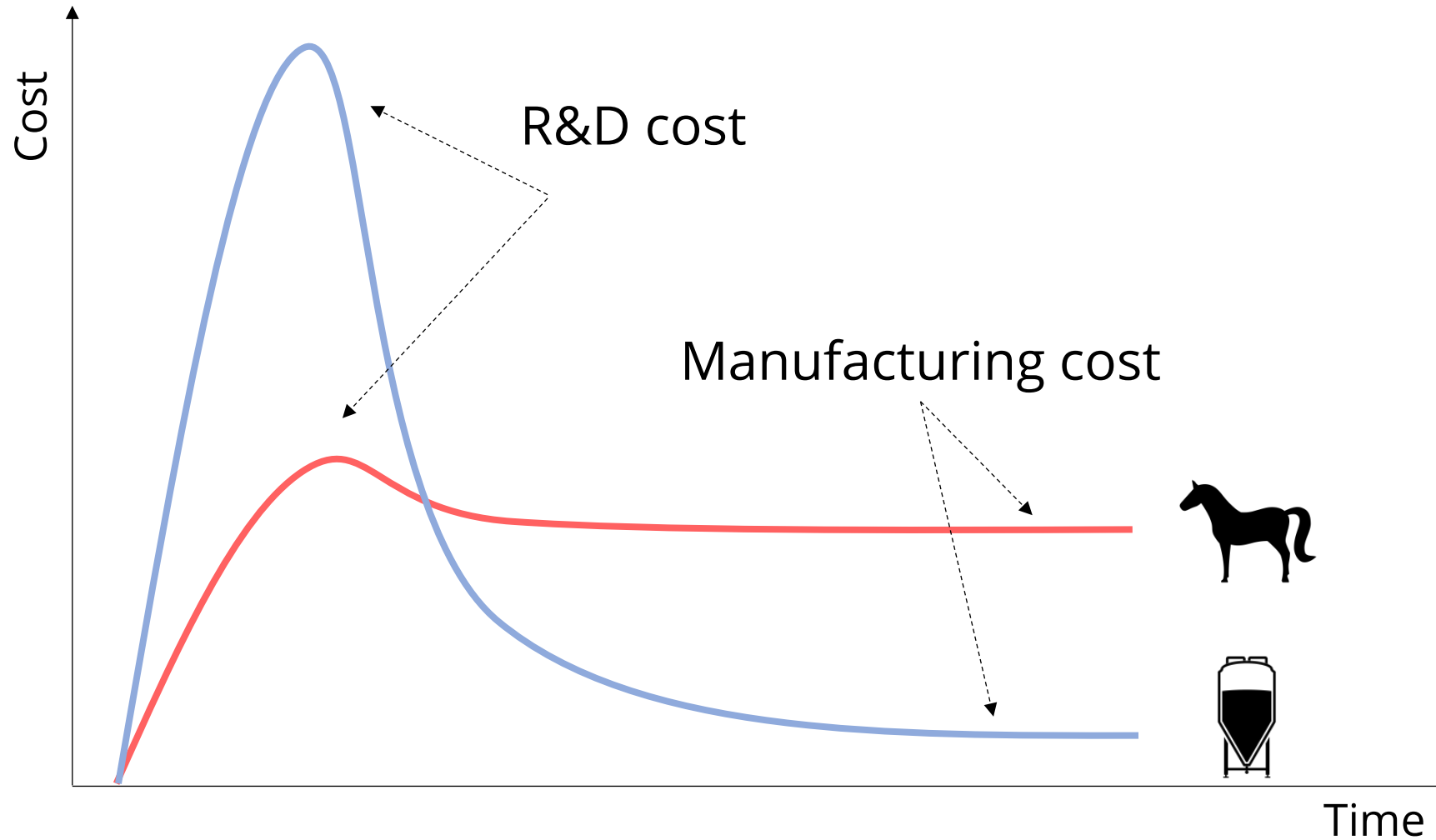
Polyvalent recombinant antivenom



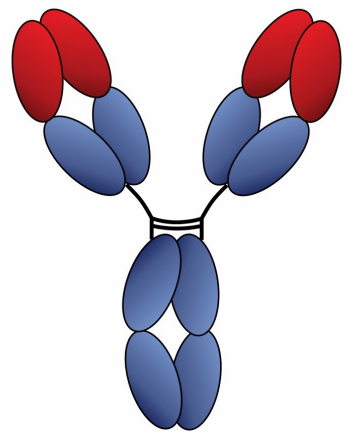




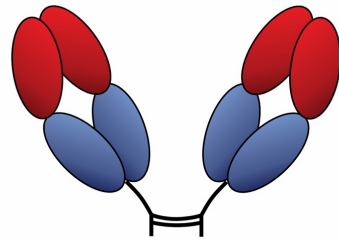
Discovery, development, and manufacturing feasibility



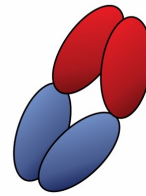
Antibody formats and their sizes



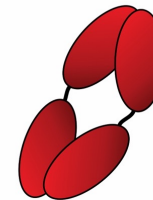
IgG
(~150 kDa)



F(ab')₂
(~110 kDa)



Fab
(~50 kDa)



Diabody
(~55 kDa)



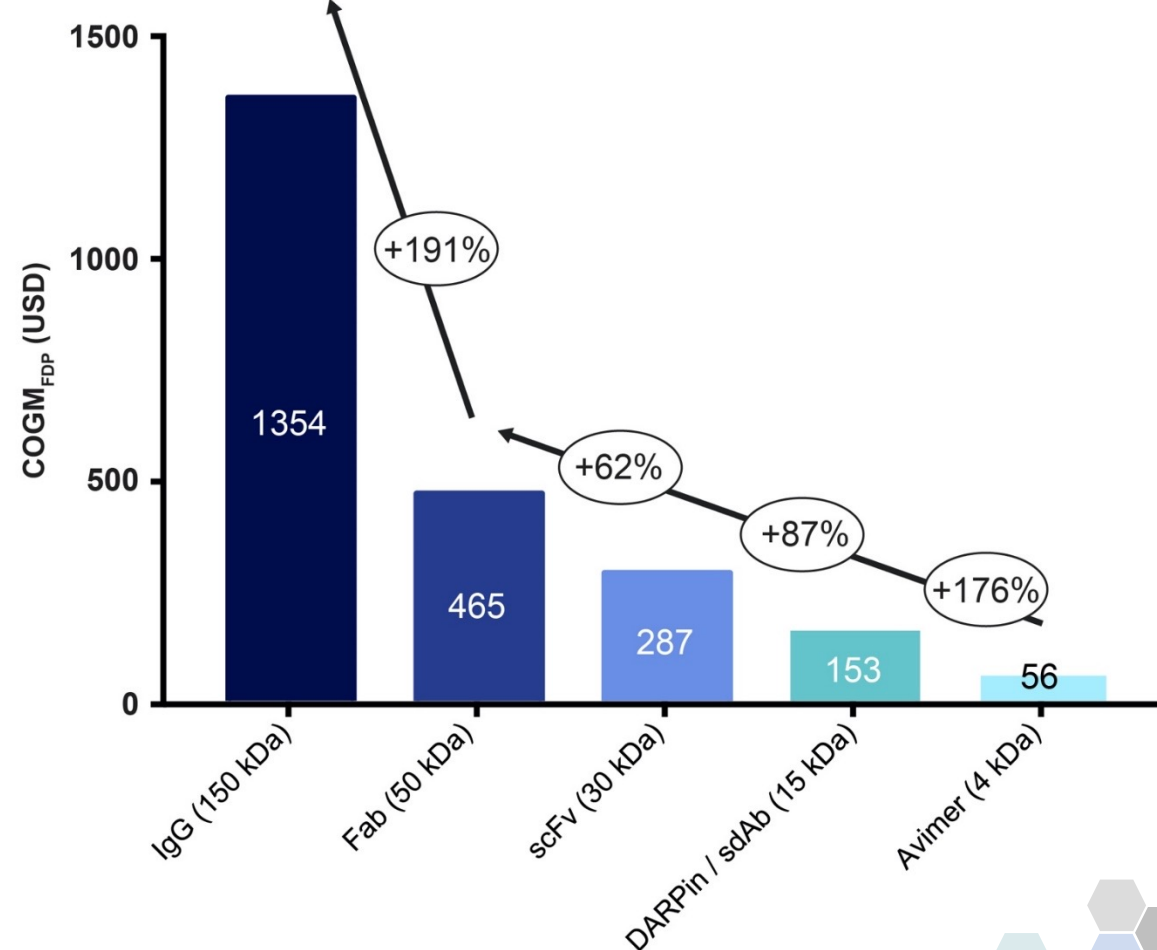
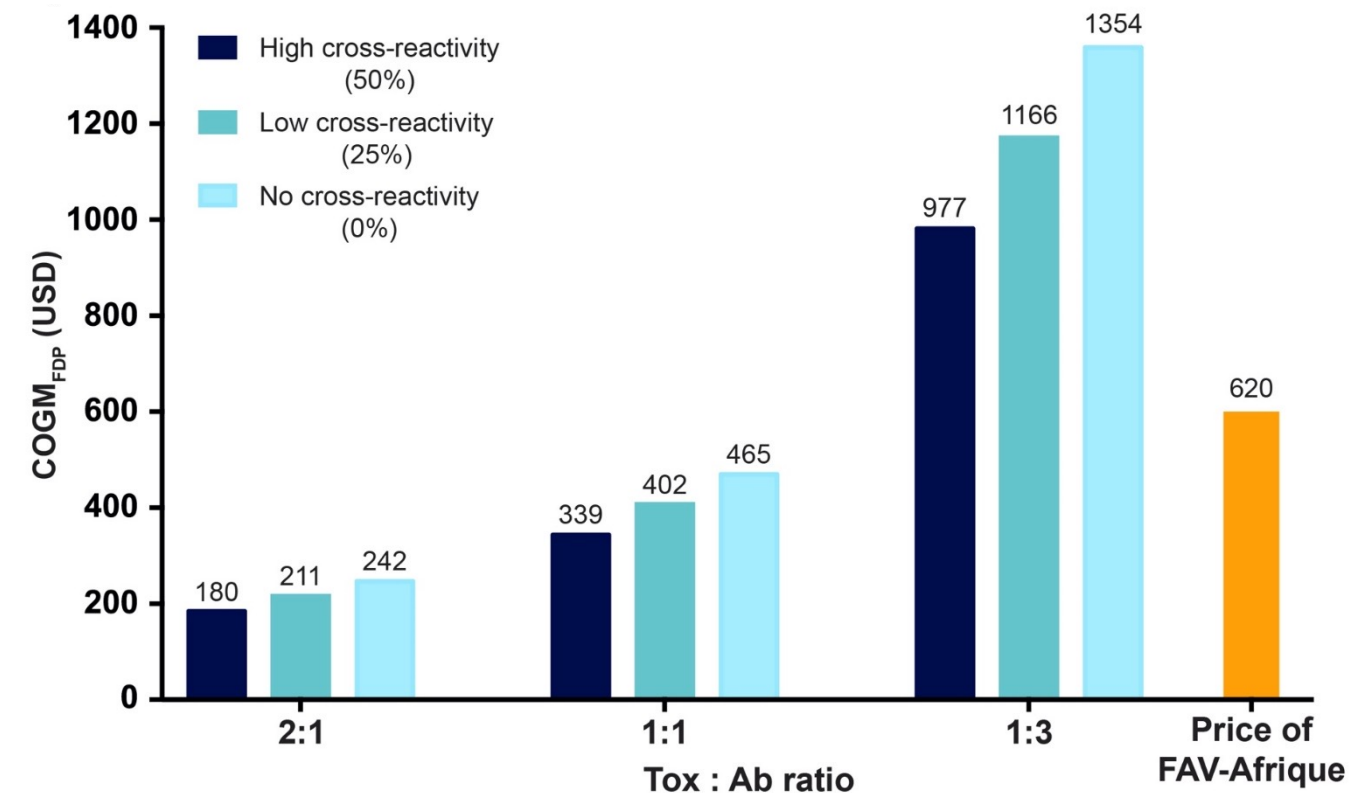
scFv
(~25 kDa)



V_HH
(~15 kDa)

Next-generation antivenoms can become affordable

African polyvalent (ten venoms)



Proof of Concept: Ultra-low-cost nanobody constructs

iScience

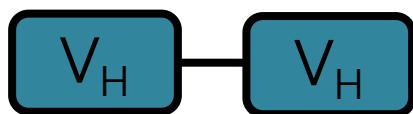
Volume 25, Issue 4, 15 April 2022, 104003



Article

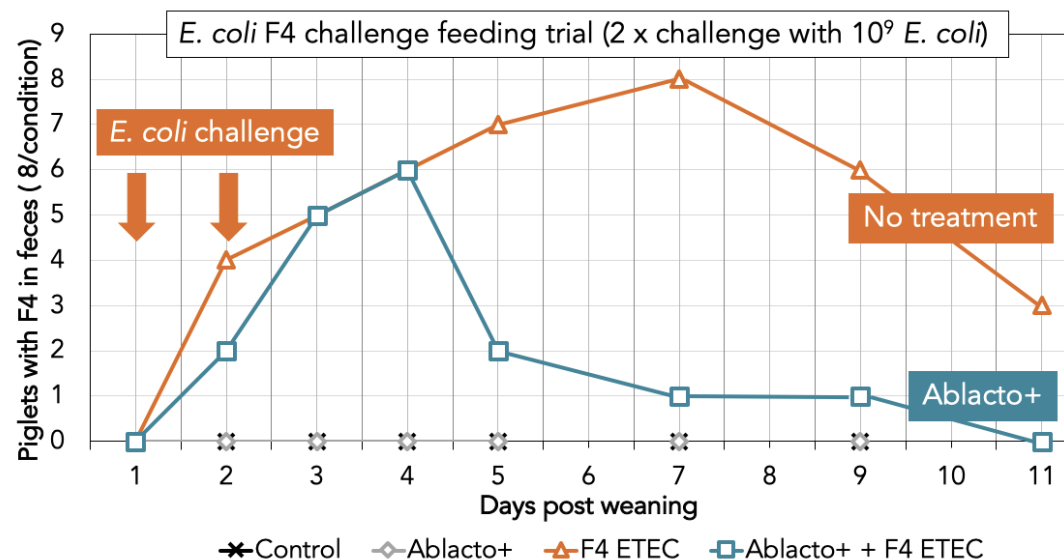
Orally active bivalent V_HH construct prevents proliferation of $F4^+$ enterotoxigenic *Escherichia coli* in weaned piglets

Berthe Katrine Fiil^{1,4}, Sandra Wingaard Thrane² ✉, Michael Pichler¹, Tiia Kittilä¹, Line Ledsgaard¹, Shirin Ahmadi¹, Grith Miriam Maigaard Hermansen^{1,2}, Lars Jelsbak¹, Charlotte Lauridsen³, Susanne Brix¹ ✉, Andreas Hougaard Laustsen^{1,2,5} ✉



Divalent constructs

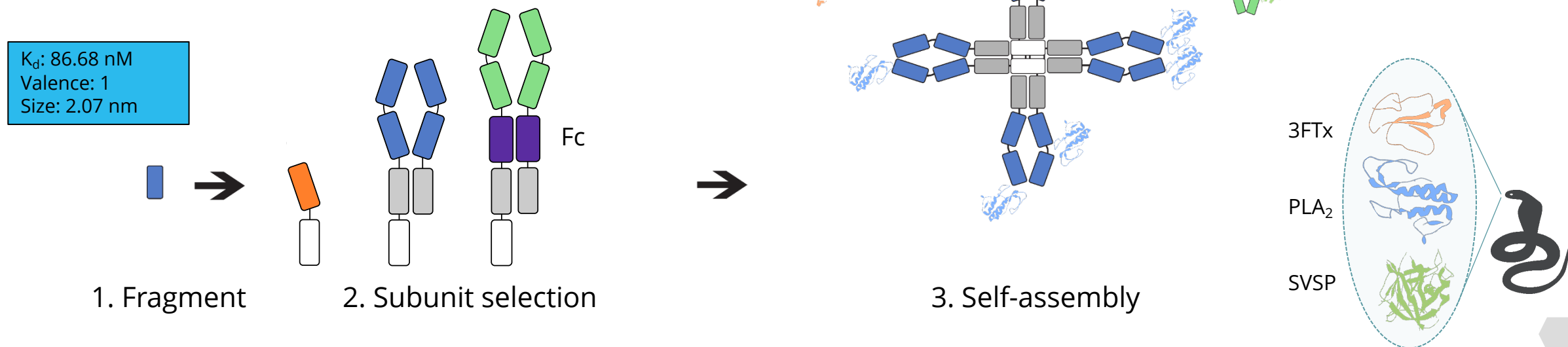
- Derived from llama hclgGs
- High affinity (K_d pM to nM range)
- pH and heat stability
- Small size (30 kDa), single-chain
- Low COGS (demonstrated in >1000 L scale)



Quadrucept technology: Low-cost, high avidity binders

Potential

- Increased avidity with improved neutralization potency
- Lower dosing
- Generate multispecific molecules
- Low cost of manufacture due to self-assembly

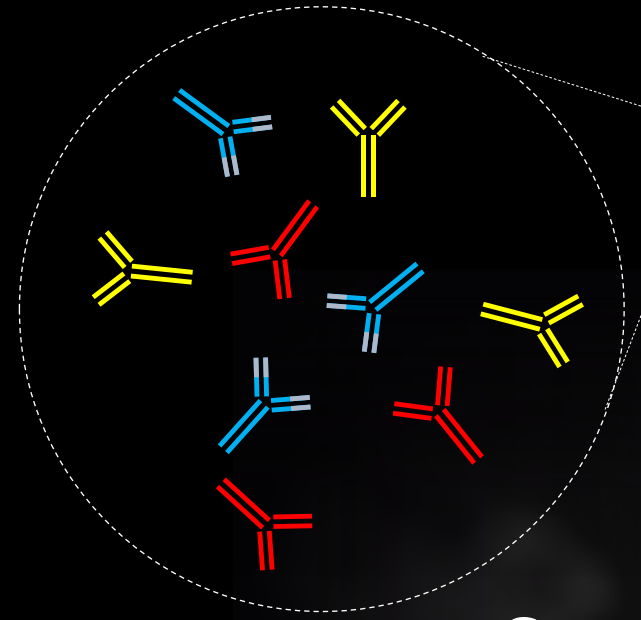
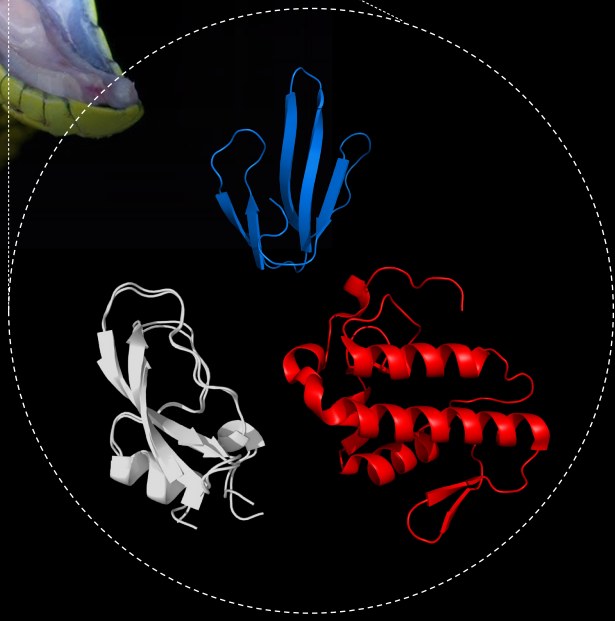




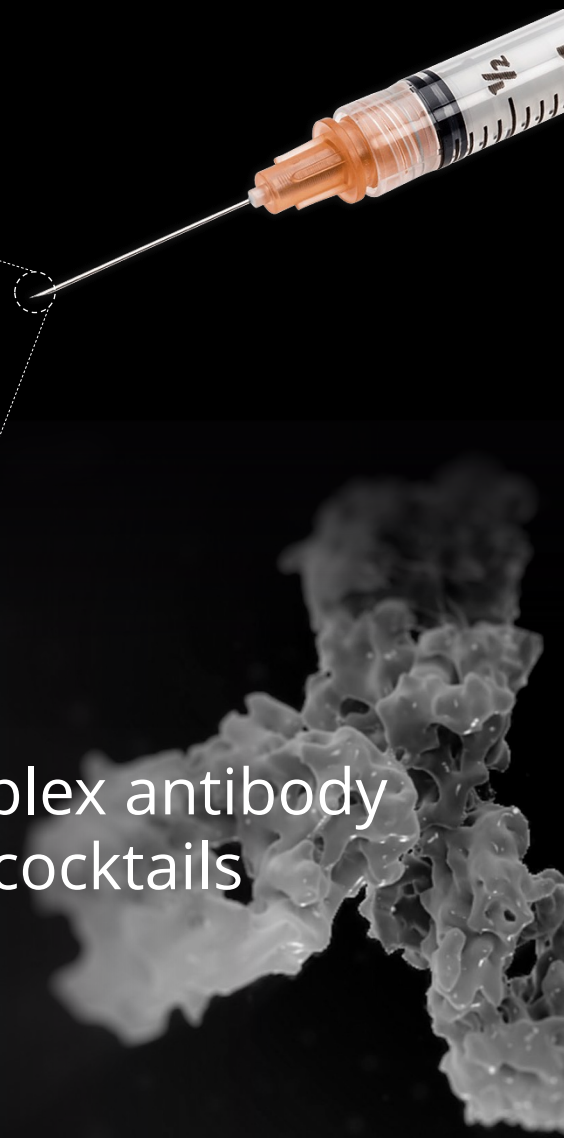
Recombinant oligoclonal broadly-neutralizing antivenom



Complex cocktails
of toxic proteins



Complex antibody
cocktails



The VenomAid DIAGNOSTICK

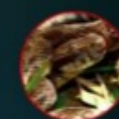
Our lead prototypes can diagnose envenomings the three medically relevant snake genera in **Brazil**:



Crotalus



Lachesis



Bothrops

These snakes are responsible for combined 99% of snakebites in **Brazil**, as well as **60,000 snakebites** annually in **Latin America**.

Future versions will allow for Point of Care use by packing the test-strips in a **plastic casing**.

CONTROL

TEST LINE

Mission
Problem
Solution
Demo
Team
Traction
Market
Business model
Finances
Vision



Thanks to



Shirin Ahmadi
Line Ledsgaard
Cecilie Knudsen
Sofie Føns
Jonas A. Jürgensen
Christoffer V. Sørensen
Urska Pus
Rahel Janke
Andrea Martos Esteban
Rasmus I. Dehli
Timothy P. Jenkins
Majken L. Olesen
Saïoa Oscoz
Albert Fuglsang-Madsen
Erick Bermúdez-Méndez
Tulika
Jack Wade
Charlotte Rimbault
Esperanza Torre
Melissa Hale
Anne Ljungars
Markus-Frederik Bohn
Frederikke A. Jeppesen
Helena H. Eriksen



Lars Ellgaard



Ulrich auf dem Keller
Farrell McGeoghan
Konstantinos Kalogeropoulos
Erwin Schoof
Jens Preben Mort
Christopher Workman
Susanne Brix Pedersen
Lars Keld Nielsen
Christina Adams
Lise Marie Grav
Lars Jelsbak
Michael Pichler
Maher A. Hachem
Marie Sofie Møller



Manuela B. Pucca



Henrik Jensen
Morten Enghave Pedersen
Nadia Mirza



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Alice M. Luther
Rachael A. Leah
Pedro Villar



CARLSBERG FOUNDATION



Brødrene Hartmanns Fond



A man with long blonde hair, wearing a white short-sleeved button-down shirt, a white fedora with a black band, and aviator sunglasses, is smiling and pointing his right index finger towards the camera. He is holding a long, thin, brown snake vertically in his left hand. The background is a sunlit outdoor area with green bushes and a dirt path.

Fangs for your attention



Antibody Engineering & Therapeutics Europe

Recombinant antivenom based on oligoclonal broadly-neutralizing antibodies

Andreas Hougaard Laustsen-Kiel

Technical University of Denmark