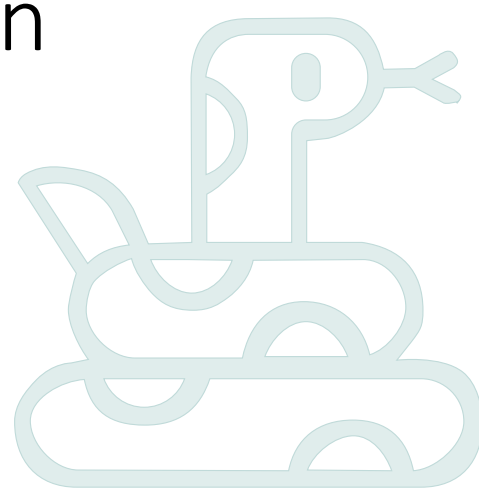
Defining Design Rules for Next-Generation Snakebite Antivenoms



Rosa webinar, 15th March 2023

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SynBio CDT

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swarm engineering across scales

UKRI Engineering and Physical Sciences Research Council

What makes a pharmacodynamically effective antivenom?

Monocled cobra
Ton Bangkeaw/Shutterstock.com

In this talk:

1. The pathology of snakebite
2. Current and next-generation antivenom production
3. Modelling envenomation and treatment
4. A framework for antivenom optimisation
5. Guidelines for effective antivenom design

Snakebite is a neglected tropical disease

Annually:

- 2.7 million envenomings
- 100,000 deaths
- 400,000 cases of disability

The burden of snakebite is overwhelmingly on developing countries

Russell's viper
RealityImages/Shutterstock.com

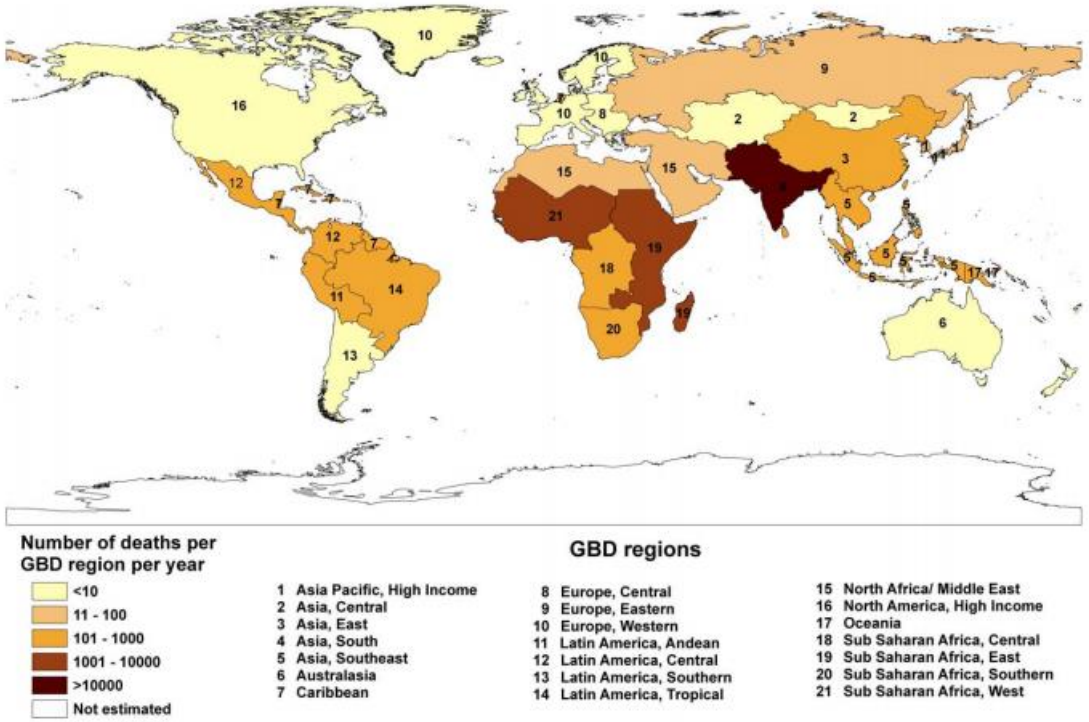
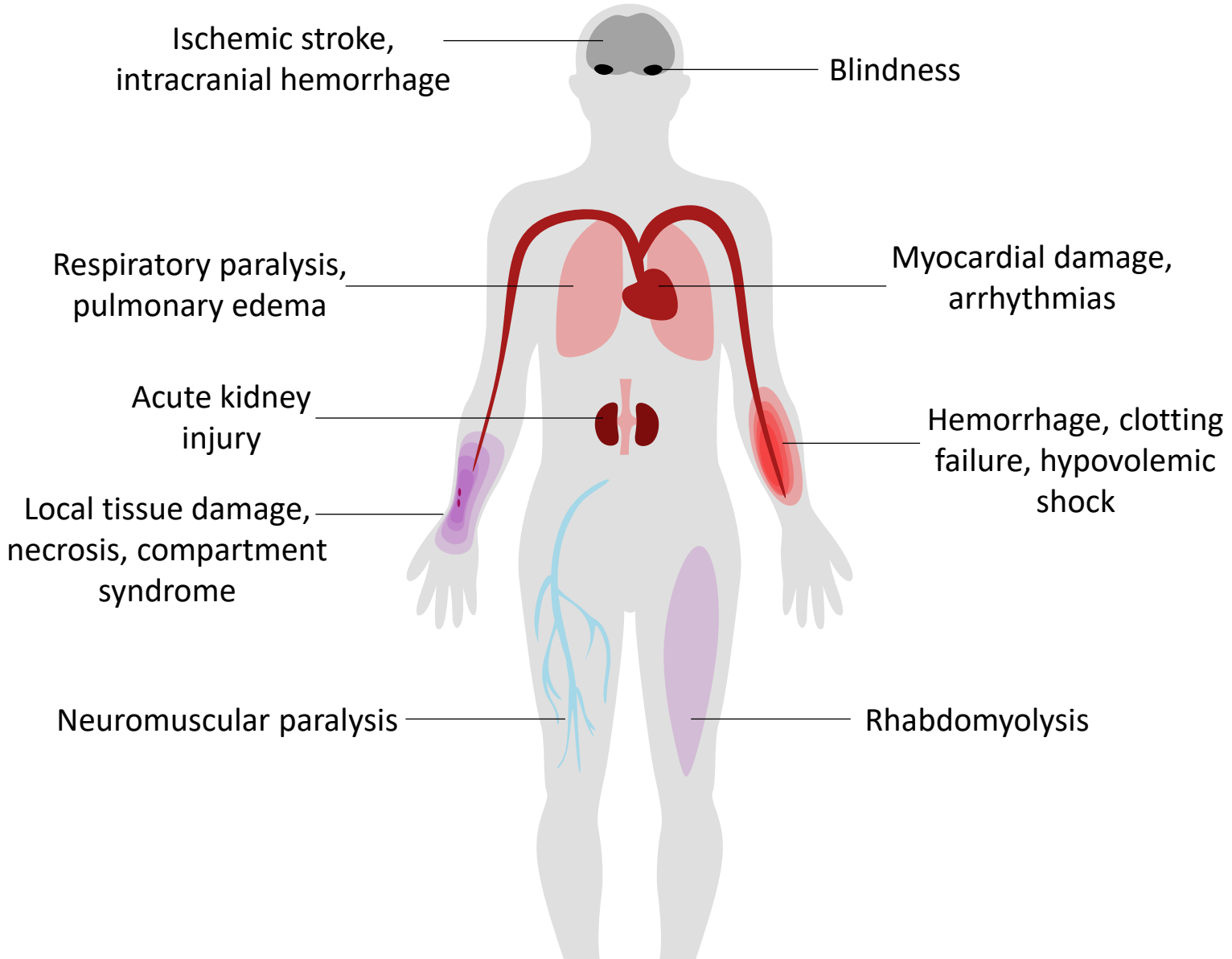


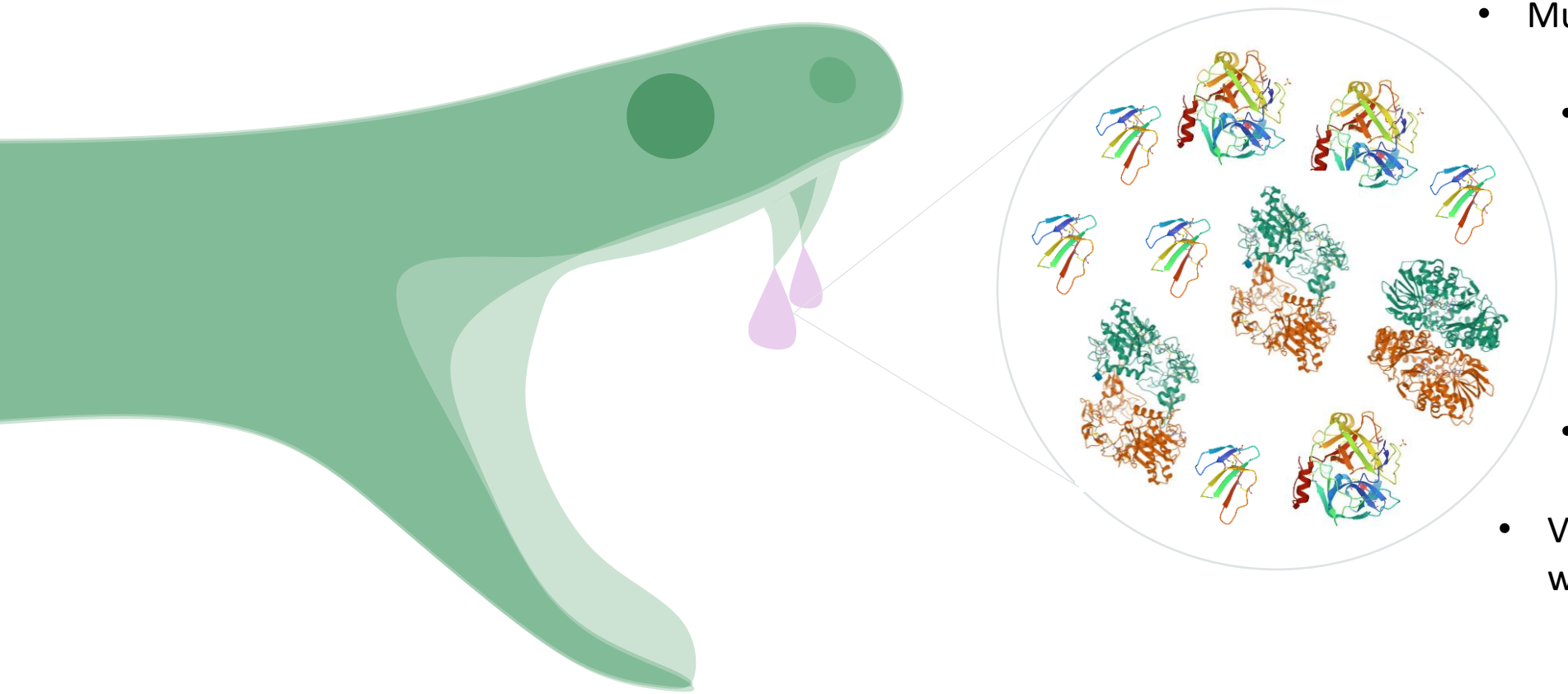
Figure 7. Regional Estimates of Deaths Due to Snakebite (Low Estimate)
doi:10.1371/journal.pmed.0050218.g007

Kasturiratne et al., 2008
https://doi.org/10.1371/journal.pmed.0050218

Snakebite causes a range of symptoms



Venom contains a complex mix of toxins



- Multifunctional
  - Local and/or systemic
    - Synergistic
    - Multiple isoforms
  - Varied structures
- Varied molecular weights: 5 – 200 kDa

Venom compositions vary between and within different species. This gives rise to diverse pathophysiological and PK properties.

There are over 200 species of medically important venomous snakes

Most of these fall into two families:

Elapids

Black mamba
NickEvansKZN/Shutterstock.com

Typically neurotoxic

- More low molecular weight toxins
- Venom more rapidly absorbs and distributes

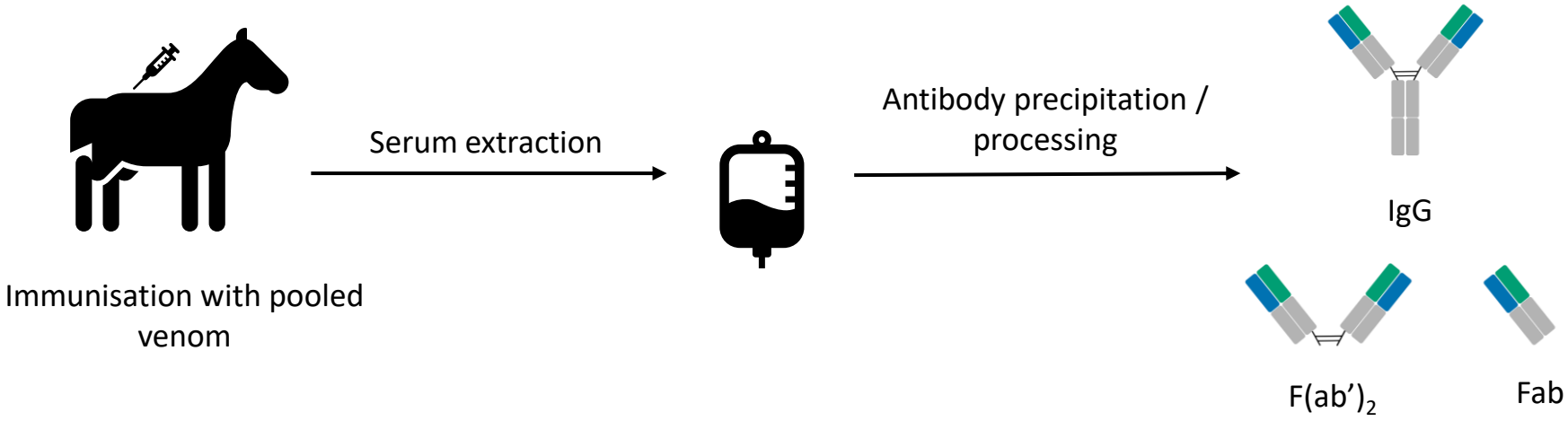
Vipers

Hump-nosed pit viper
RealityImages/Shutterstock.com

Typically haemotoxic and cytotoxic

- More high molecular weight toxins
- Venom absorbs more slowly and persists for longer

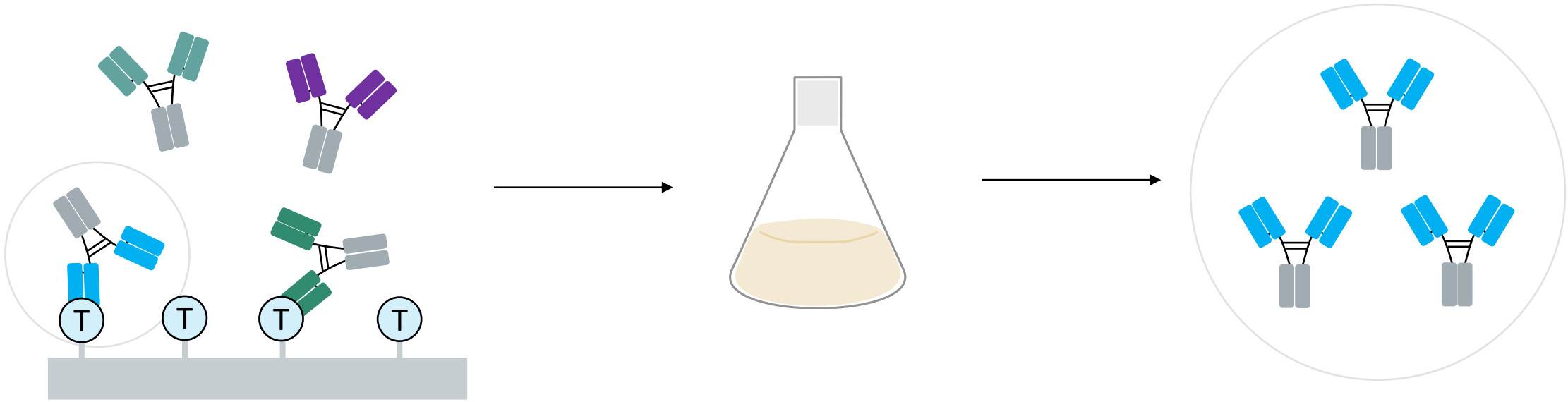
Antivenoms are currently made from the sera of hyper-immunized animals



Limitations:

- Expensive
- Low therapeutic potency
- Batch variability
- Ineffective against necrosis
- High risk of adverse effects
- Requires animal husbandry

Next-generation recombinant antivenoms



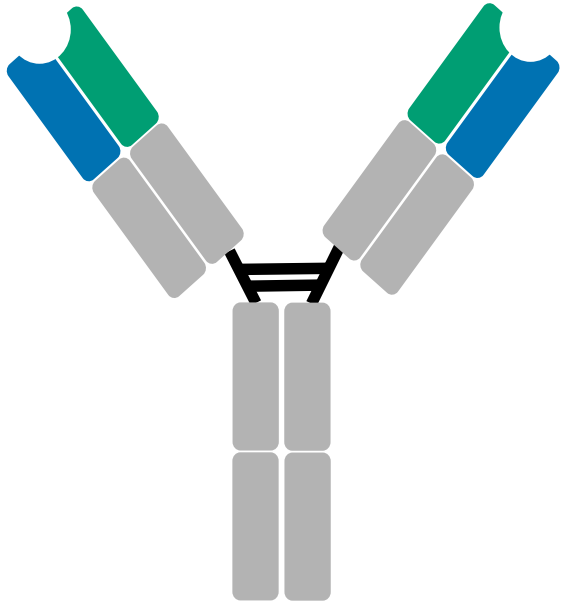
***In vitro* selection**
Obtain toxin-binding antibodies from variable library

Recombinant expression
Produce best antibodies in cellular culture

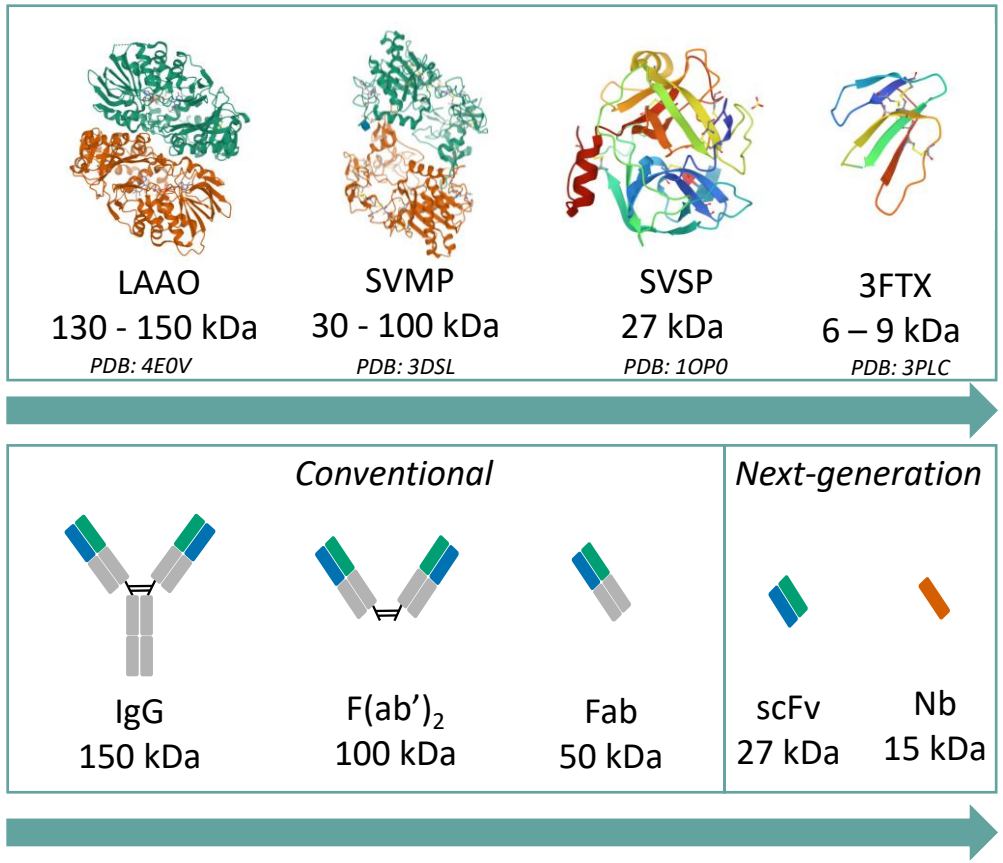
Recombinant antivenoms
Targeted binders

Antibody engineering has expanded antivenom design space

- ***In vitro* selection** → scaffold type
- **Antibody humanisation** → immunogenicity
- **Affinity maturation** → affinity
- **Structural engineering** → valency, size, half-life



We can produce antivenoms with diverse PK/PD properties



Antivenom scaffolds span a similarly wide size range to venom toxins themselves

Decreasing size: Increasing elimination rate, increasing tissue perfusion

How does antivenom format affect treatment outcome?

Elapid

Rapid absorption
Rapid distribution
Faster elimination

Viper

Slower absorption
Slower distribution
Longer persistence

Computational simulations can help elucidate venom-antivenom pharmacodynamics

- Are certain scaffolds better suited to treat different types of venoms?
- Are certain scaffolds preferable under particular envenomation scenarios?

We simulated two model venoms

Ton Bangkeaw/Shutterstock.com

Elapid – Equatorial spitting cobra

- Low molecular weight (9kDa)
- Neurotoxic
- Rapidly and extensively distributes

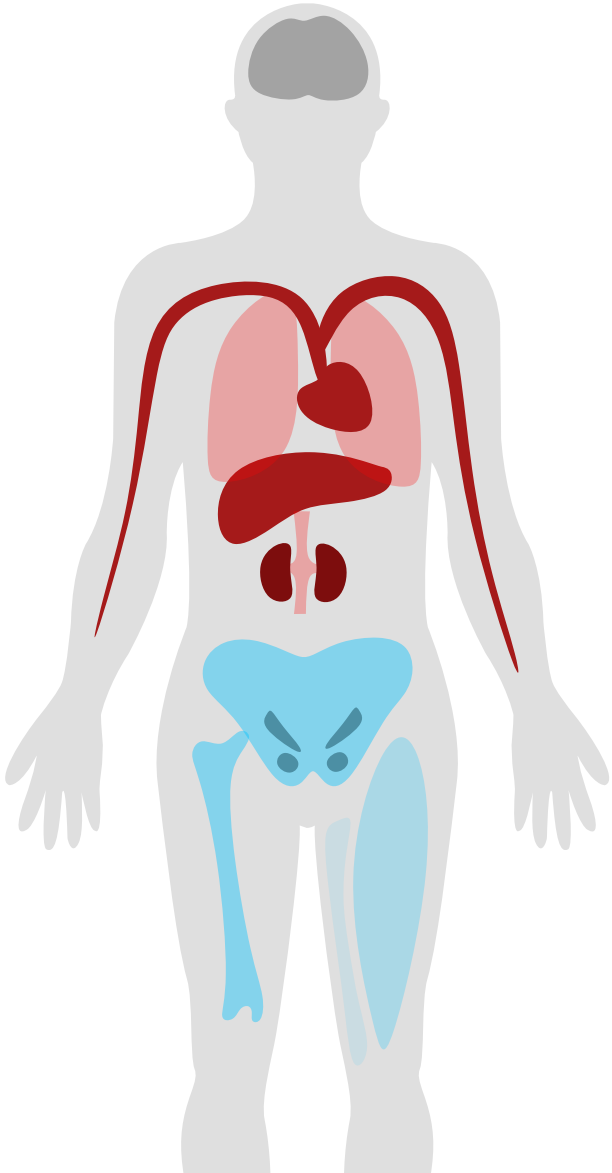
Kurit Afshen/Shutterstock.com

Viper – Mangrove pit viper

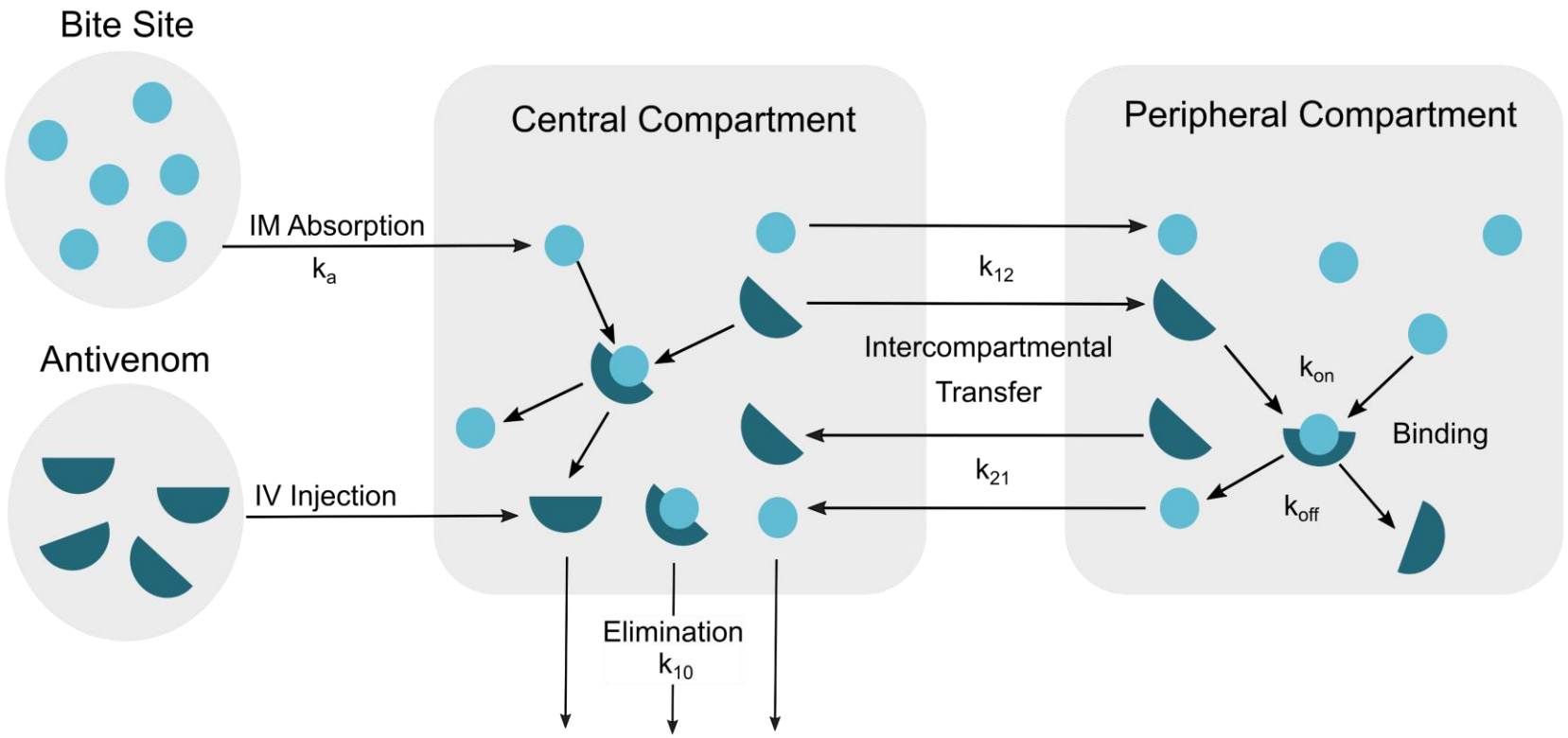
- High molecular weight (57kDa)
- Haemotoxic
- Distributes slowly, longer half-life

Why compartmental modelling?

- Describes bulk system dynamics through central and peripheral compartments
  - Indicates lethality
  - Granular description
- Can be parameterized with existing venom/antivenom data
- Simple and computationally efficient
  - Fewer parameters
  - Brute force parameter optimisation
  - Can map parameter space to high resolution



The compartmental model

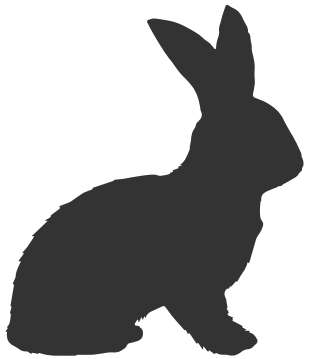


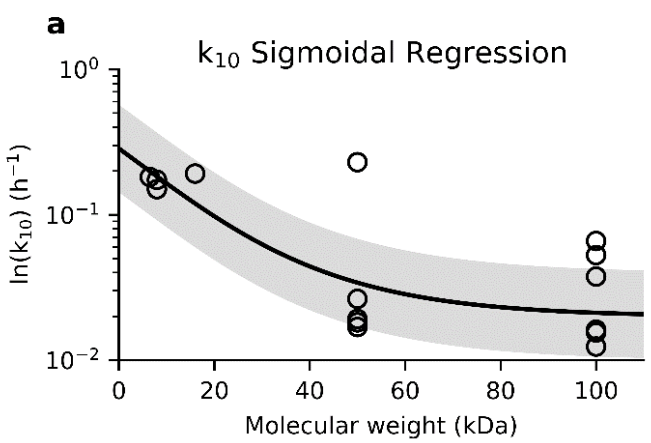
- Body split into central and peripheral compartments
- Following the levels of venom, antivenom, and neutralised venom
- Monovalent and bivalent binding

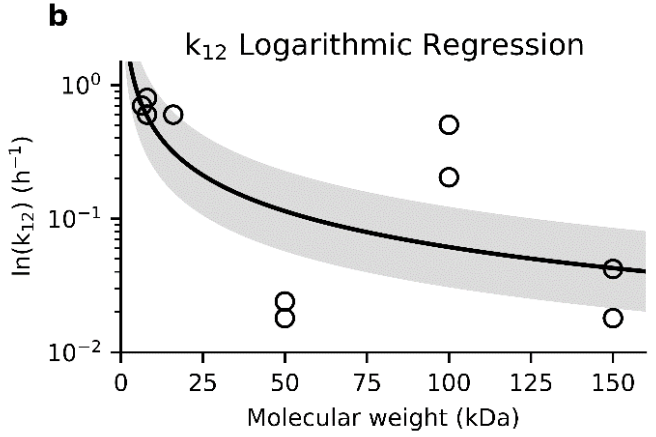
Model parameterisation

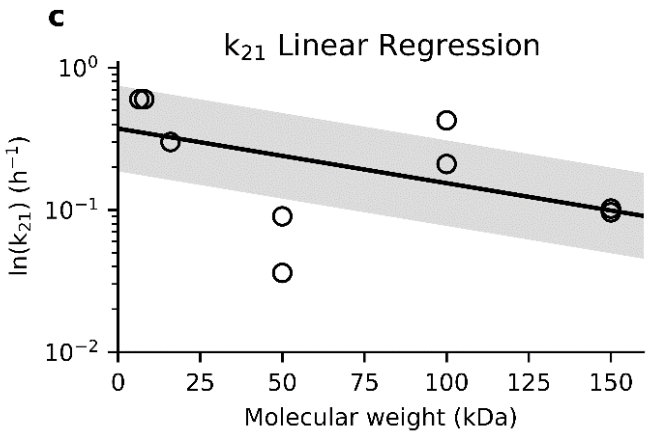
Model parameterised using experimental rabbit data

- Venom parameters taken directly from literature
- Antivenom and neutralised venom parameters predicted based on molecular size using regressions



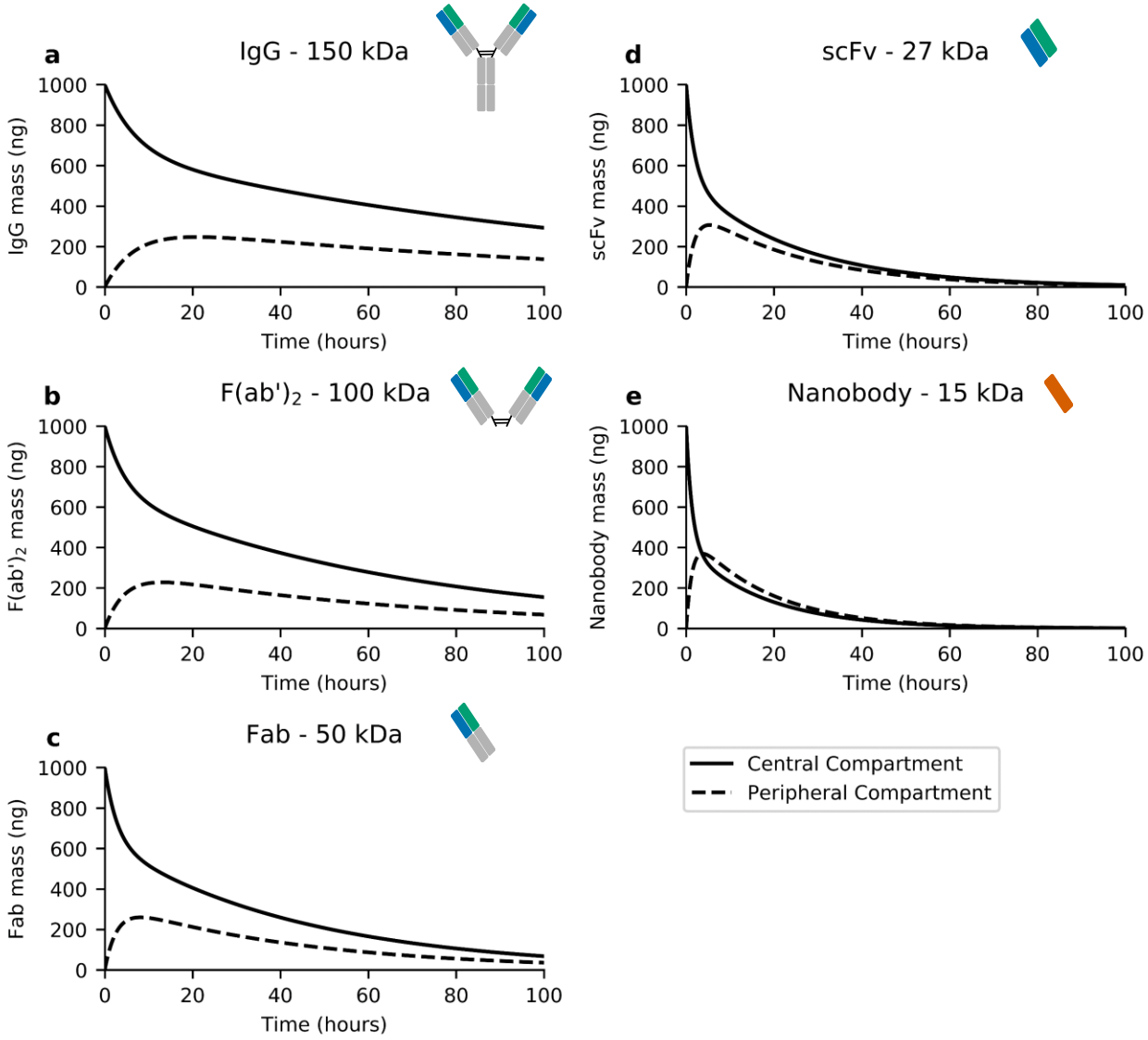




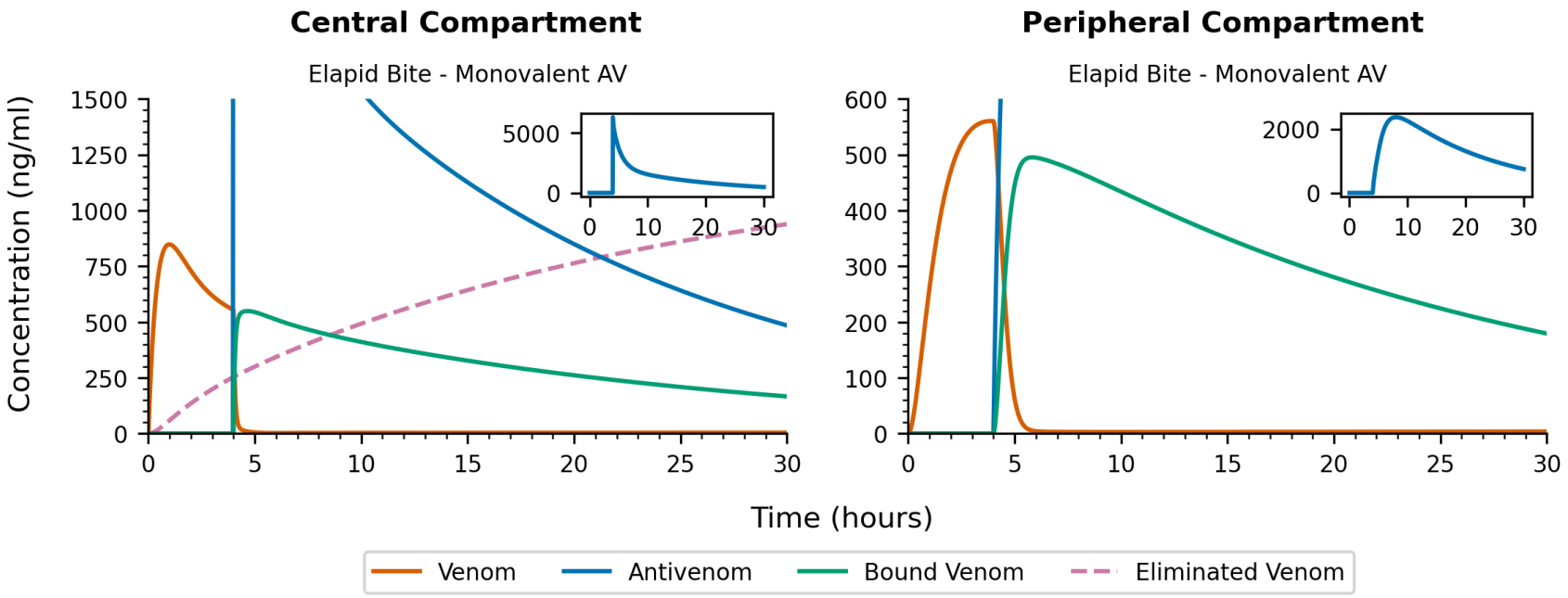


Predicting antivenom dynamics

- Antivenom $k_{10}/k_{12}/k_{21}$ parameters predicted based on molecular size using regressions



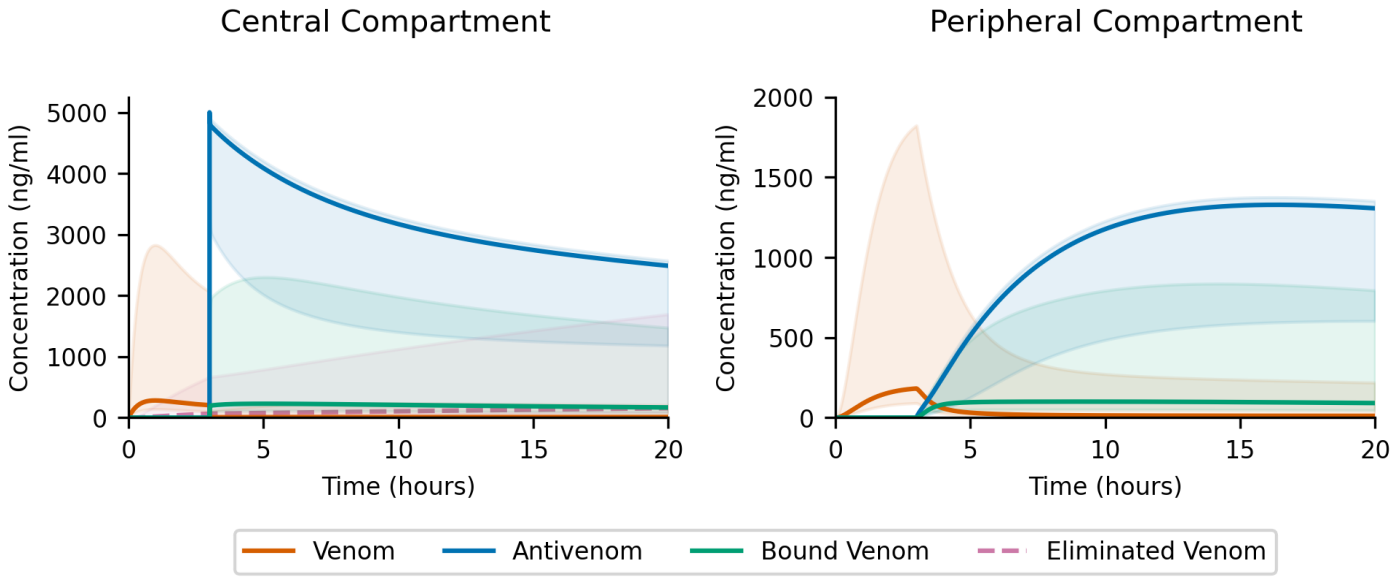
The model allows user-control of numerous parameters



- 3 mg elapid venom
- Treat at 4 hours
- Monovalent nanobody
- 1:3 venom: antivenom dose
- $k_{on} = 1x10^{4}\ M^{-1}\ s^{-1}$
- $k_{off} = 1x10^{-5}\ s^{-1}$

Simulating variable envenomation scenarios

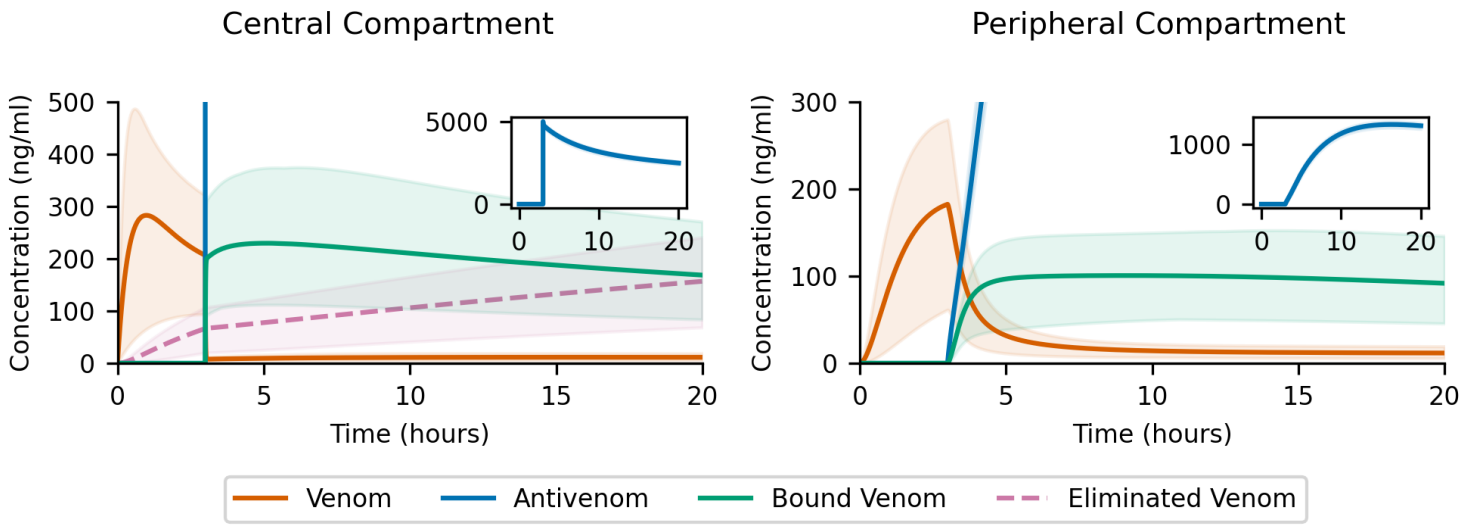
Snakes can inject variable amounts of venom:



- Applying an elapid venom dose range of 0.25 – 5 mg/kg
- Treat with 2.5 mg/kg $F(ab')_2$ antivenom

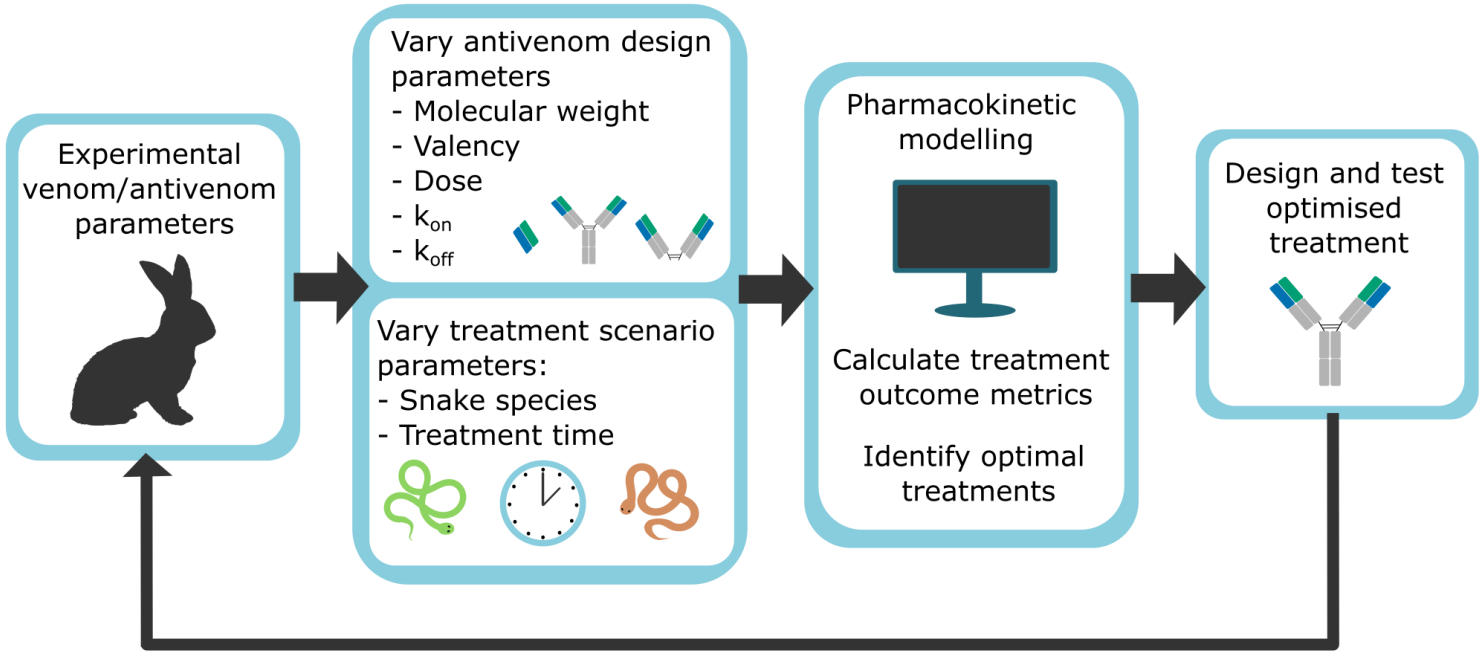
Simulating variable envenomation scenarios

Snakes can bite in different locations and to different depths:



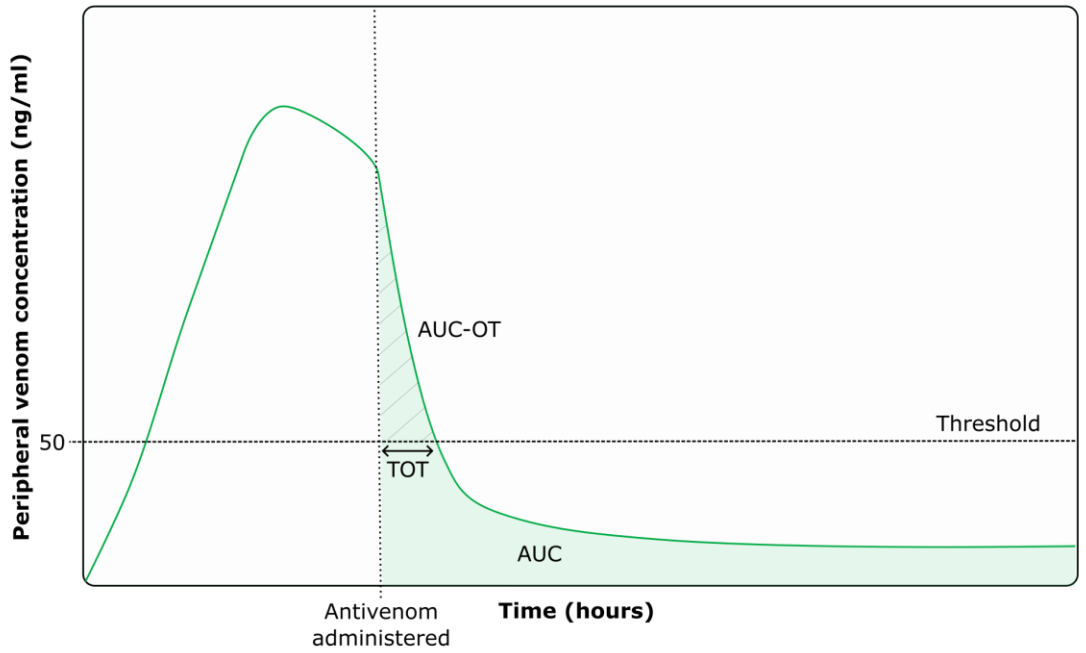
- Applying 0.5 mg/kg elapid venom
- Treat with 2.5 mg/kg $F(ab')_2$ antivenom
- F varies +- 50% over baseline
- Absorption rate varies: T_{max} from 0.5 – 3 hours

A framework for antivenom optimisation



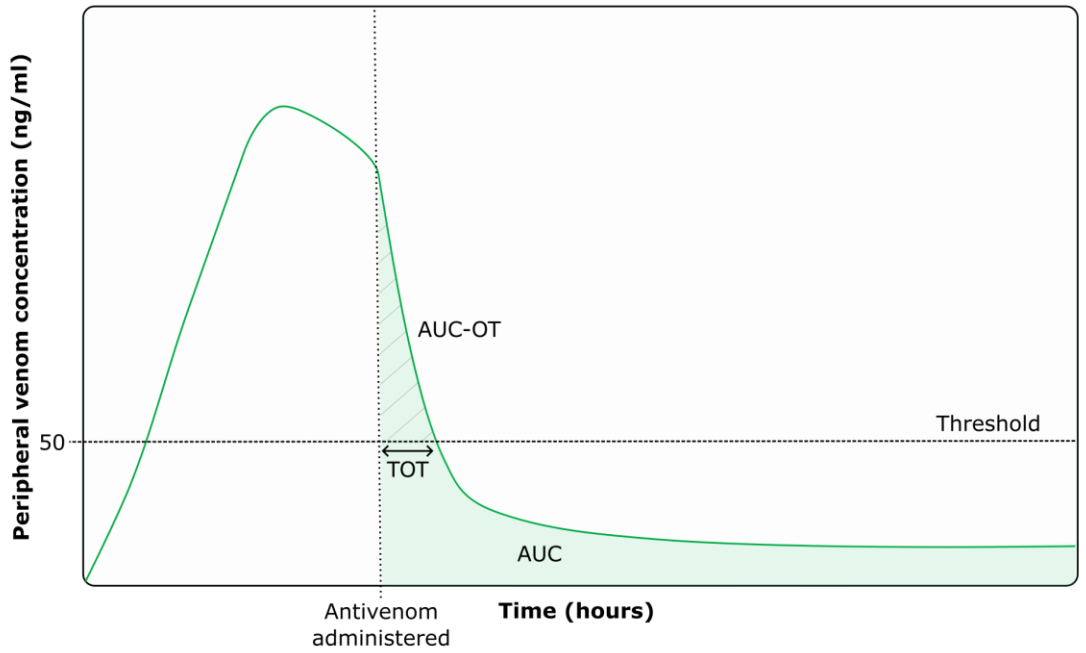
Defining treatment metrics

- We looked at three metrics to indicate damage:
  - Area under the curve (AUC)
  - Time over threshold (TOT)
  - AUC over a threshold (AUC-OT)



Defining treatment metrics

- We looked at three metrics to indicate damage:
  - Area under the curve (AUC)
  - Time over threshold (TOT)
  - **AUC over a threshold (AUC-OT), applied to peripheral compartment**
- Threshold informed by clinical envenoming studies

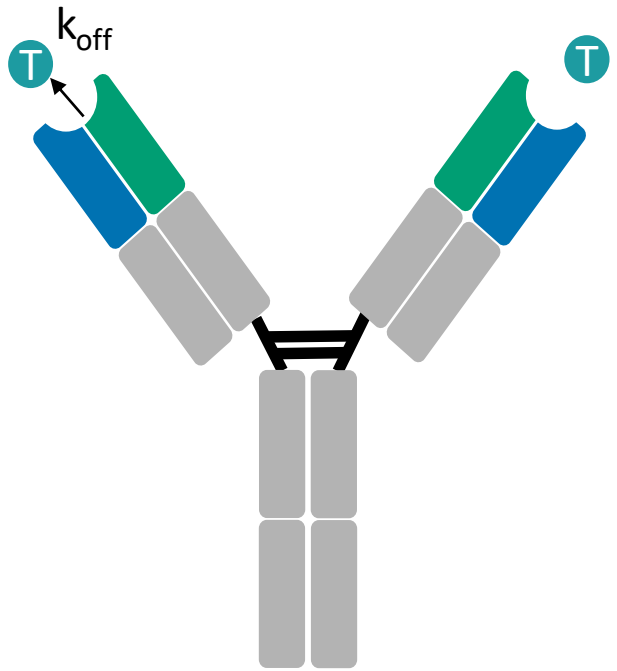


Defining the antivenom parameter set

We generated a set of 200,00 theoretical antivenoms, which varied across 5 dimensions:

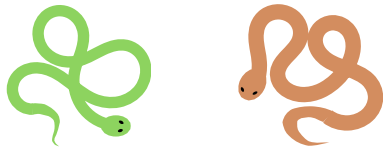
- **Molecular weight** – 15 – 150 kDa
- **Valency** – 1 or 2
- **k_{on}** - 10^{3} - 10^{6} $M^{-1}s^{-1}$
- **k_{off}** - 10^{-6}- 10^{-3} s^{-1}
- **Dose** – 1:1 – 1:10

15 kDa



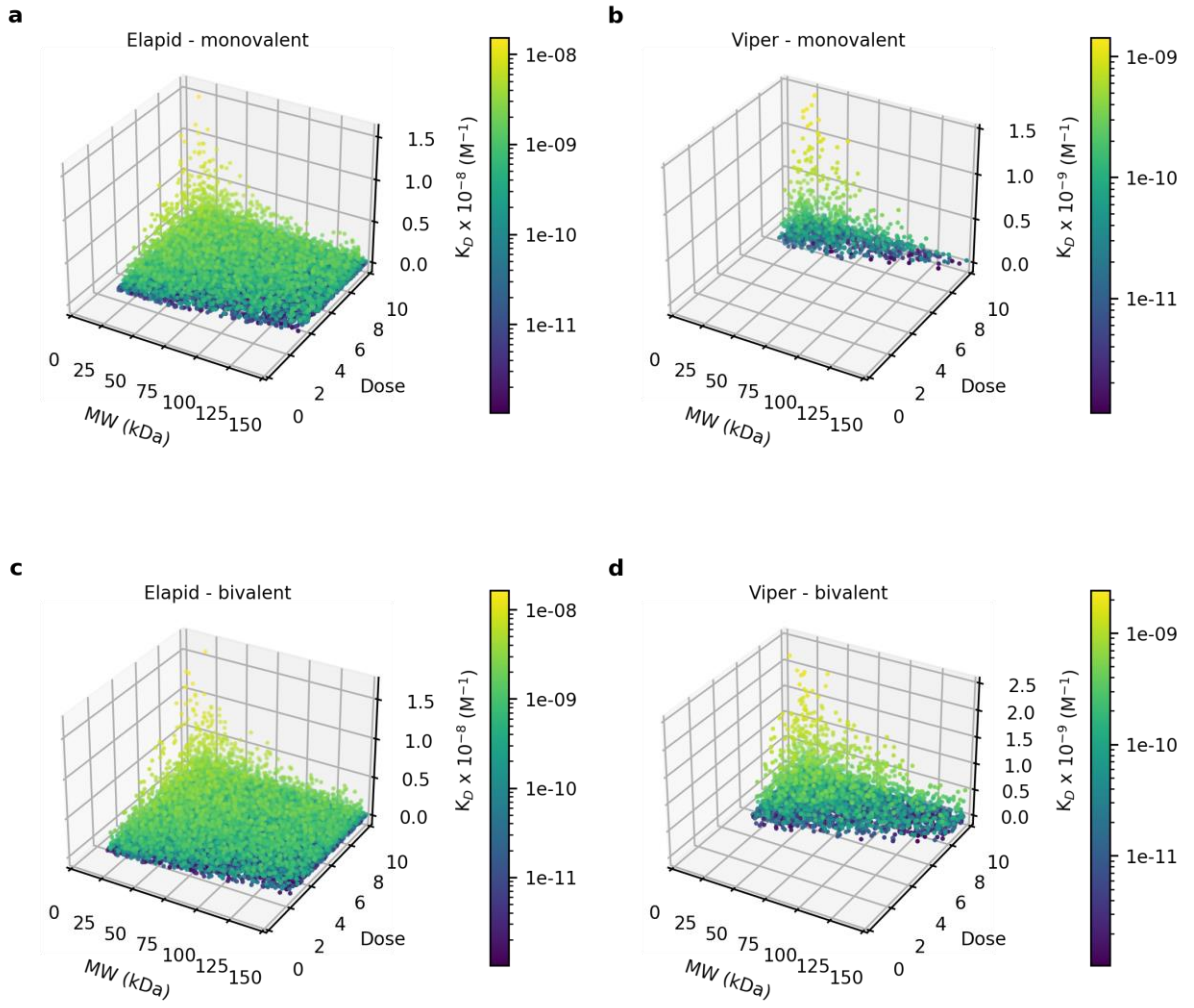
150 kDa

Varying treatment scenario parameters

- Comparing **elapid** and **viper** envenomation 
- Simulated **treatment times** ranging hourly from 1-10 h post bite 
- **Total of 2 million simulations per snake**

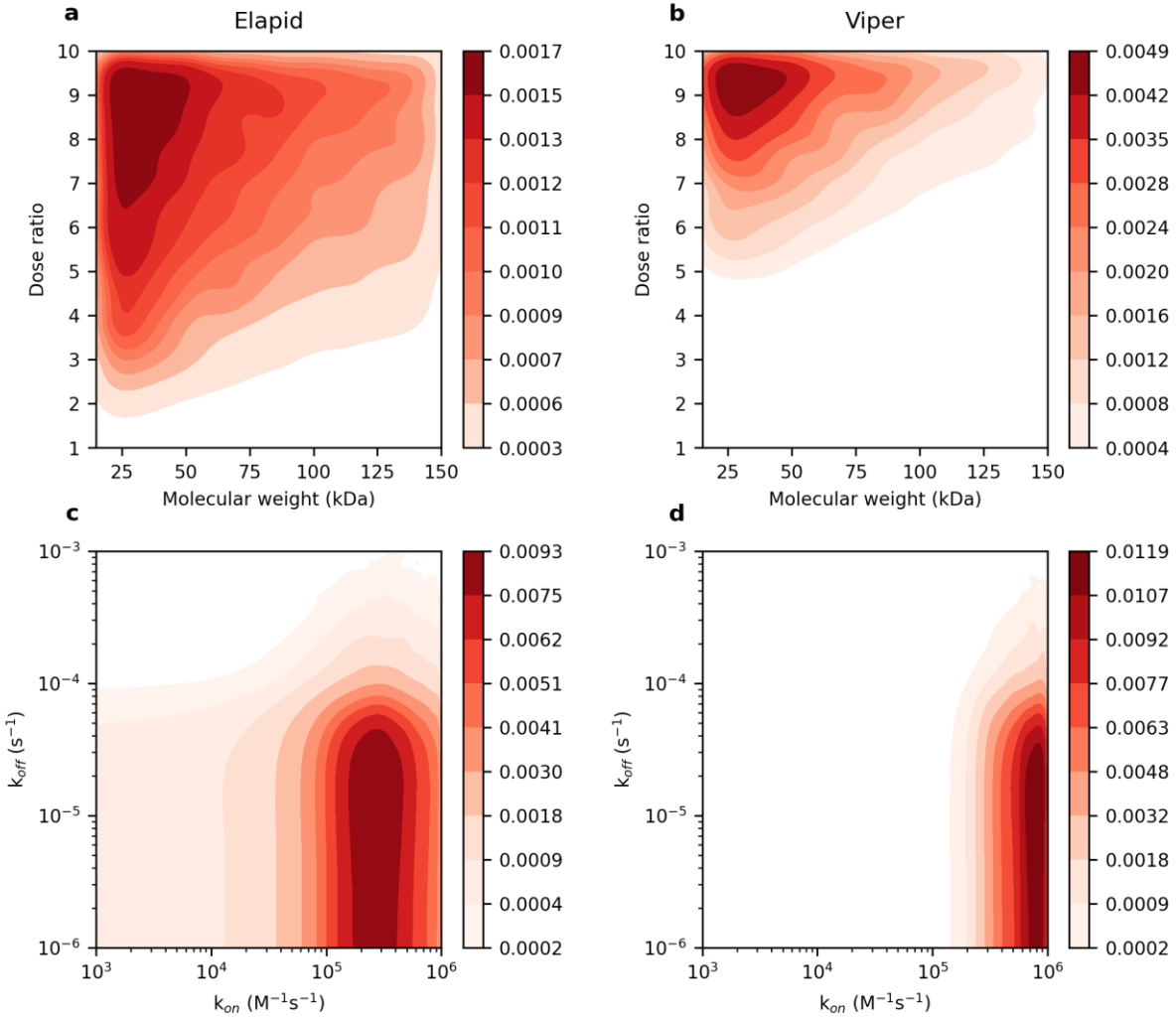
Universal scaffolds

- Antivenoms with lowest 1% AUC-OT at every timepoint
  - High affinity
  - High dose
  - Tolerant of molecular weight & valency
  - More stringent design constraints for viper bite



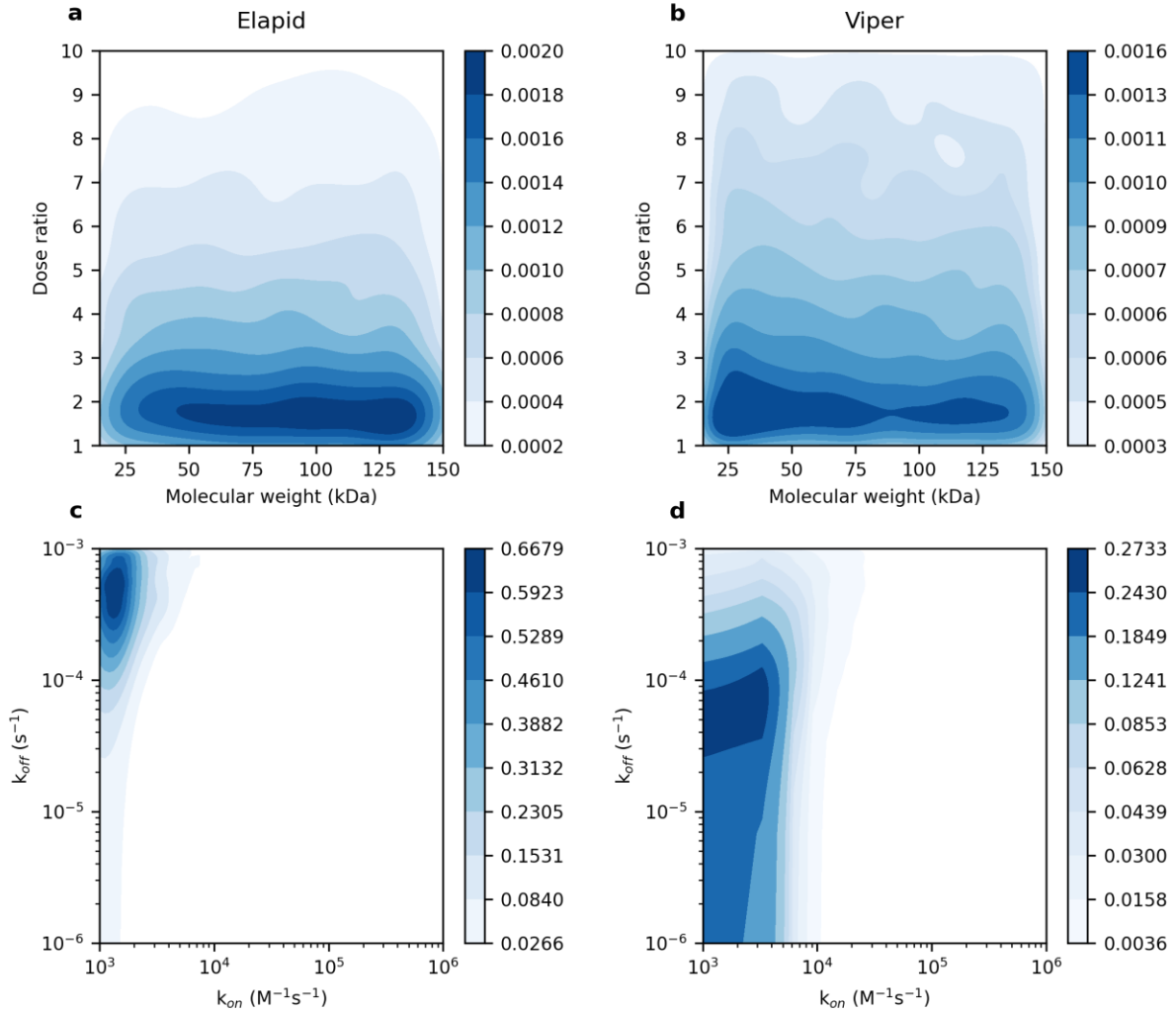
Universal scaffolds

- Antivenoms with lowest 1% AUC-OT at every timepoint
- Density across parameter space
  - Preference for low molecular weight
  - Preference for high k_{on}



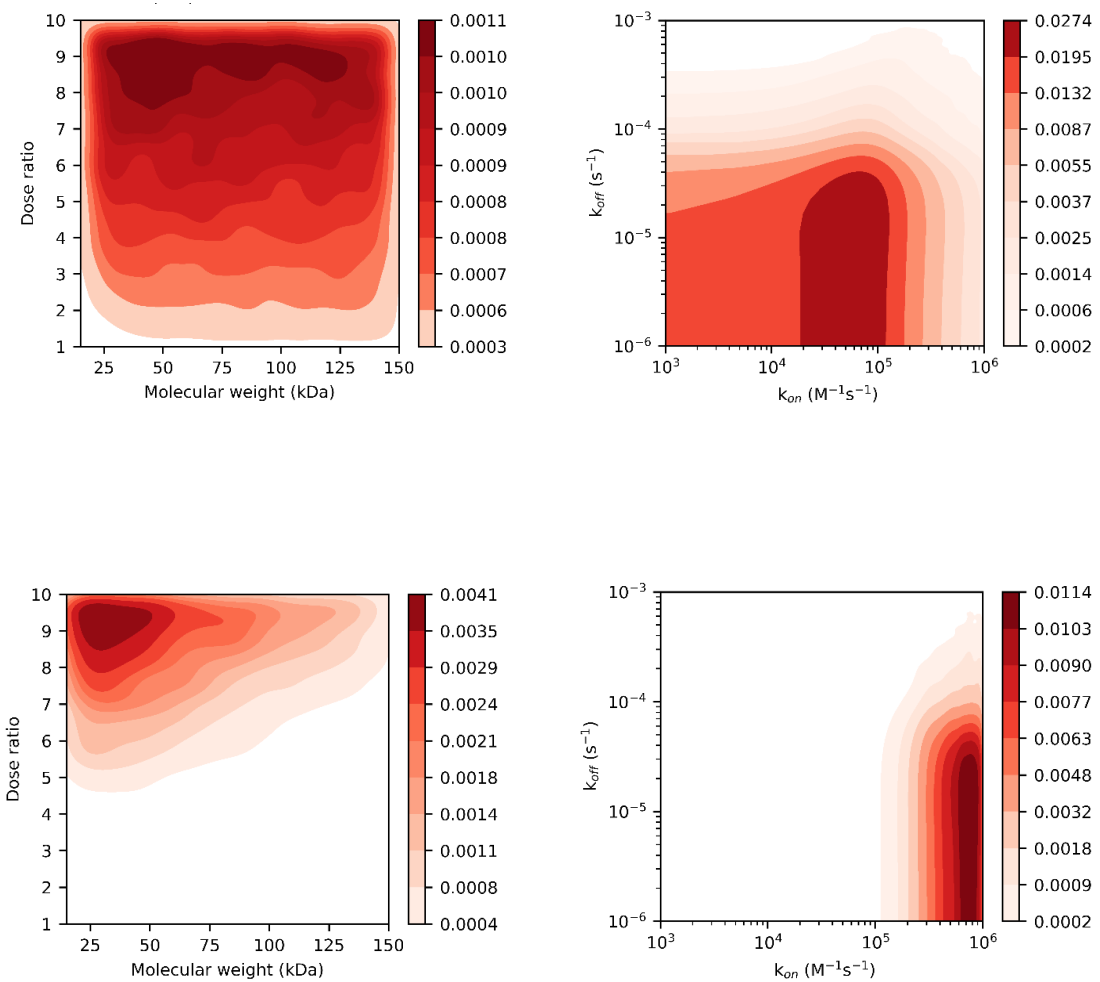
Poorly-performing antivenoms

- Parameter space of antivenoms with highest 50% AUC-OT at every treatment time
- Density across parameter space
  - Low dose, low k_{on}
  - Poor performers across the size range

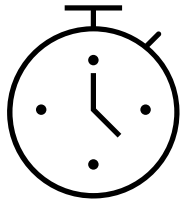


Time-dependent variations

Viper scaffolds with lowest 1% AUC-OT with different time delays

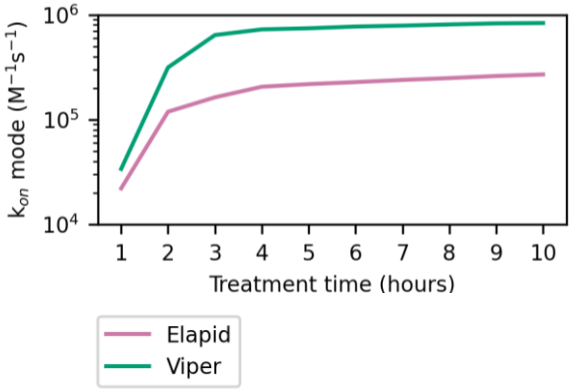


Antivenom at 1 hour

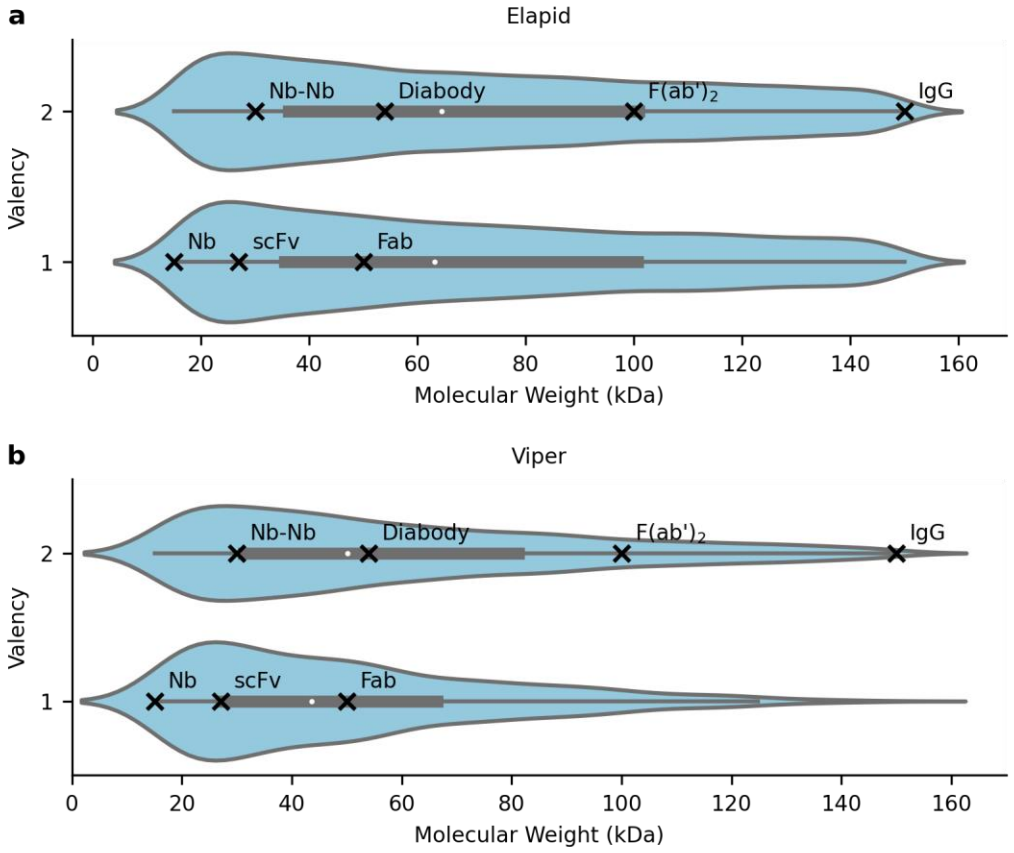


Antivenom at 6 hours

Preference for smaller size and high k_{on} with increasing treatment delays



Visualizing the most effective scaffolds

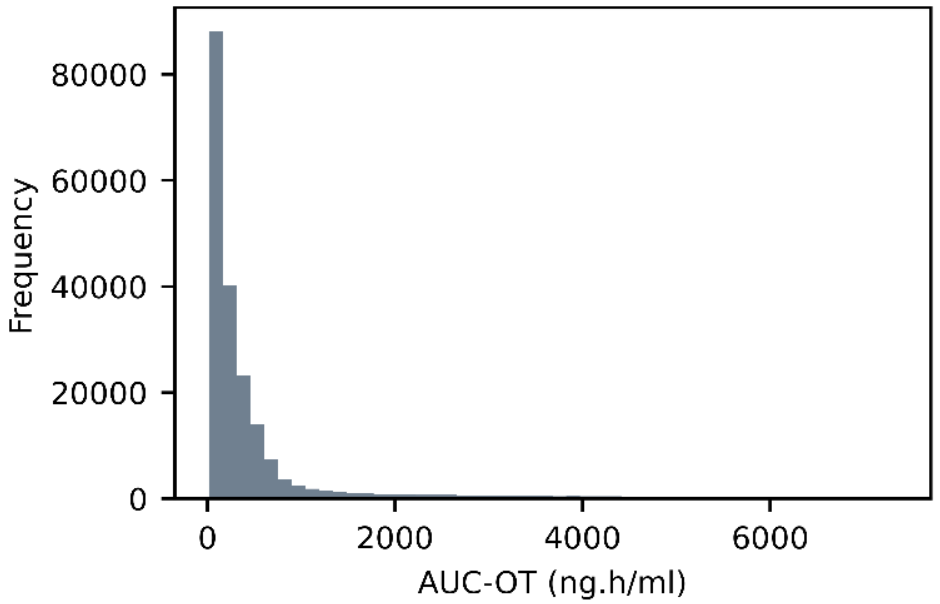


- Violin plots of universal scaffolds at every timepoint
- Smaller scaffolds offer the most flexible design constraints
  - More effective scaffold solutions found at lower molecular weights

PAWN global sensitivity analysis

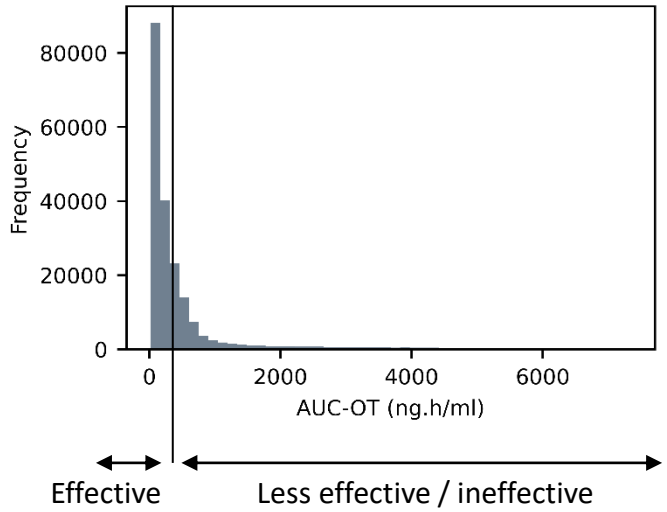
- Density-based GSA method
  - Good for highly skewed outputs
- What design parameters influence treatment outcome the most?
- Sensitivity indices indicate the influence of a given parameter on a model output
  - **Bigger index = bigger influence**

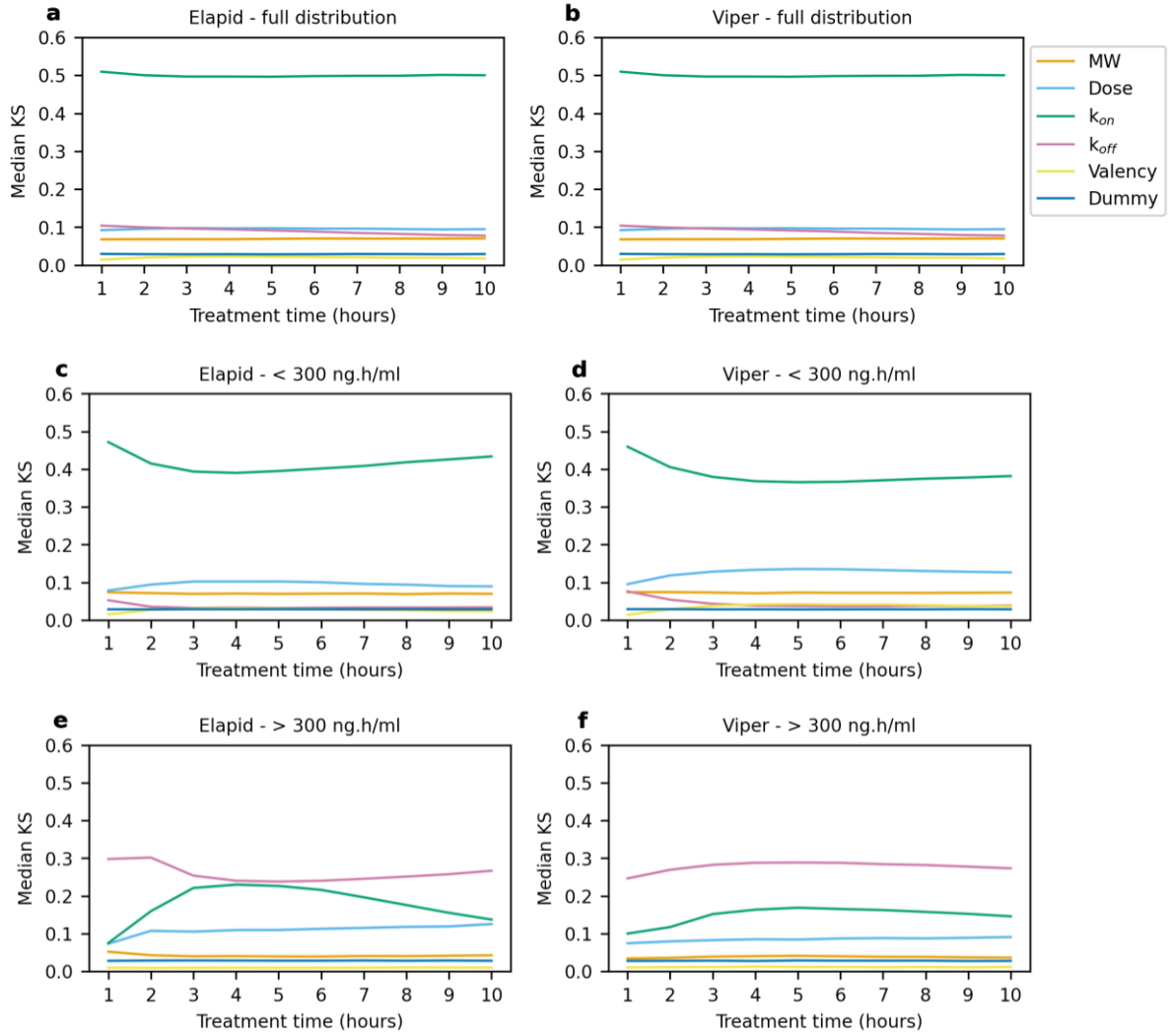
Example model output – elapid, 3 hours



PAWN sensitivity analysis

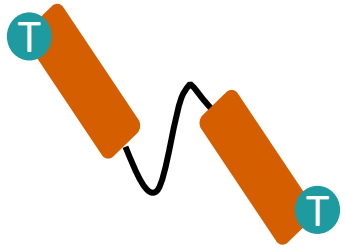
- Dummy parameter sets threshold of influence
- Testing how sensitivity changes over time
- **Looked at the full output distribution**
  - k_{on} most important overall
- **Looked at slices of the distribution**
  - k_{off} has a bigger impact on poorly-performing antivenoms





Guidelines for effective antivenom design

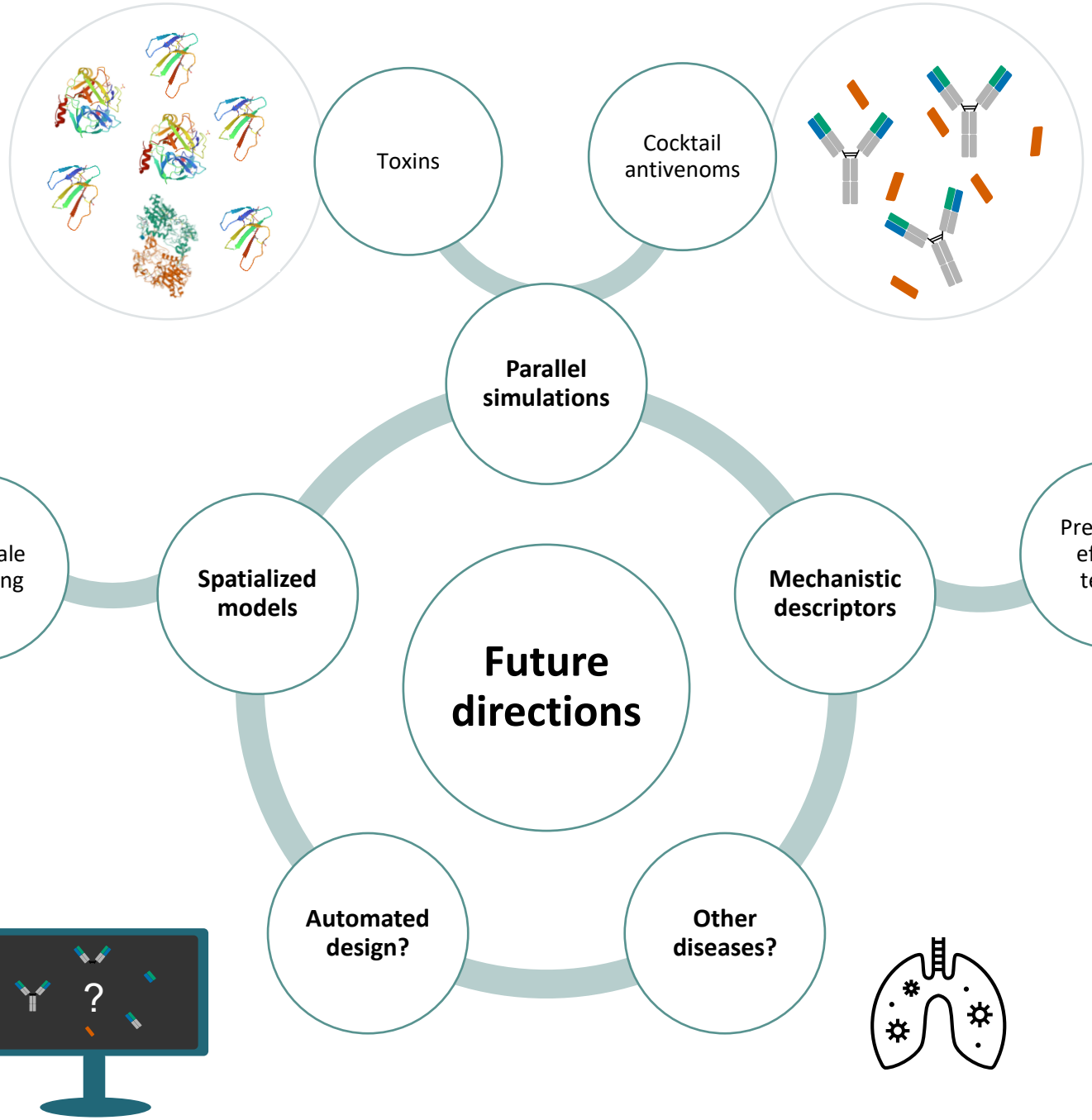
1. Optimised antivenoms can span a **wide area of design space**
2. Treatment outcome **primarily mediated by affinity (k_{on})**
3. **Size has a minimal direct impact, but small scaffolds can be more flexibly designed**
4. **Higher doses are better**. Small scaffolds out-perform larger scaffolds when dosed sufficiently.
5. **Viper and elapid** systems are optimally treated by the **same types of scaffold**



Bivalent nanobody
30 kDa

$k_{on} > 10^5\ M^{-1}s^{-1}$
$k_{off} < 10^{-4}\ s^{-1}$

Monovalent scFv
27 kDa

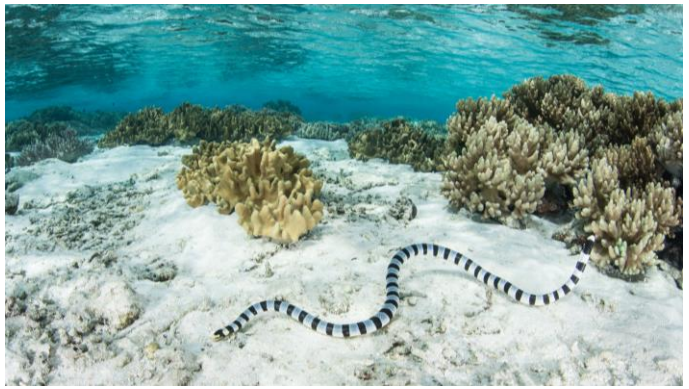

Future directions
Parallel simulations
Toxins
Cocktail antivenoms
Mechanistic descriptors
Pre-clinical efficacy testing
Other diseases?
Automated design?
Spatialized models
Multiscale modelling
?

Summary

1. Venom and antivenom pharmacodynamics is complex
2. We have built a computational model of systemic snakebite envenomation and treatment
3. It is parameterised to allow user-control of antivenom size, affinity, valency, dosing schedules, and venom type
4. We have established a computational framework to optimise antivenom design
5. Parameter optimisation shows that antivenom affinity is key. Molecular size doesn't have a huge direct impact, but smaller scaffolds allow for more flexible treatment

Mangshan pit viper
Henner Damke - stock.adobe.com

Western green mamba
Vencav - stock.adobe.com



Banded sea krait
Ead72 - stock.adobe.com

Thanks for listening!

Special thanks to Sabine Hauert, Johanna Blee, and all members of the swarm engineering group

Feel free to contact me at: natalie.morris@bristol.ac.uk

All code (Python) available via the below publications

HAUERT LAB
swarm engineering across scales

SynBio CDT

University of BRISTOL

UKRI Engineering and Physical Sciences Research Council

Morris et al, 2022. Developing a computational pharmacokinetic model of systemic snakebite envenomation and antivenom treatment**.** Toxicon, 215, pp. 77-90. https://doi.org/10.1016/j.toxicon.2022.06.006.

Morris et al, 2023. Global parameter optimisation and sensitivity analysis of snake antivenom pharmacokinetics and pharmacodynamics. bioRxiv. doi: https://doi.org/10.1101/2023.03.13.532354

Painted saw-scaled viper
AbuMazna/Shutterstock.com

Mozambique spitting cobra
Stu Porter/Shutterstock.com

Boomslang
Petra Christen/Shutterstock.com