Tab 1

Toxbind – De novo antivenom minibinders using BindCraft

About

This is a repository for the snake venom binder project using BindCraft. The project is based on the paper "De novo designed proteins neutralize lethal snake venom toxins" at Baker lab. The paper describes the design of de novo anti-venoms that can neutralize the 3FTx family of snake venom. Here the approach varies in the methods for generating the binder. The paper is based on the RFDiffusion-based method. Here, we are using BindCraft, as it can create high-quality binders without using a lot of sampling. It has become an effective method for binder design in the community.

Introduction

Snakebite envenoming remains a devastating and neglected tropical disease, claiming over 100,000 lives annually and causing severe complications and long-lasting disabilities for many more. India alone constitutes half of the mentioned death number and disabilities, and known as the snakebit capital of the earth. Around the world, antivenom is the main form of treatment, the production of antivenom has remained unchanged over a century. In India, a polyvalent antivenom is produced for the treatment of envenomations from ‘big four’ snakes. However, there are many venomous snakes which we don’t have antivenom for, producing customized antivenom for each and every species is virtually impossible. Also, the venom profile of snakes is also dependent on climate and the prey they feed upon. So, it has been seen that even venom from the same species varies between different geographies. Most of the snake bite cases happened at the lower end of the society i.e. farmers working on the crop fields, people sleeping on the ground during nights. Because of lack of the proper treatment and lack of accessibility of the antivenom in adequate time leads to even more severe cases. Also, current antivenom needs to be kept at a cool temperature and requires refrigeration. Therefore, it can’t be extended and kept at the primary healthcare facilities. As the risk of climate changes increases, the conflict between snake and human cases will continue to increase. Also, because of more and more forests cut and converted to farming, more pressure is put on wildlife survival, leading to forage of food beyond its territories leading to more such cases.

Current Challenges

One more aspect of the current polyvalent antivenom is efficacy itself, only a certain fraction of antivenom contains neutralizing antibodies, when depending on severity of bites, it might be required to administer more antivenom vials. However, given that it is animal derived, it causes allergic reactions in most humans to some extent, prolonging medical care at the hospitals.

Problem with animal-derived polyclonal antibody

- Composition of immunogen will vary
- Variable immune response of animal → Variable composition of antibody cocktail
- Only a fraction of injected antibodies "relevant"
  - Directed to non-venom targets
  - Venom components unrelated to toxicity
- Low antibody titers against highly toxic but poorly immunogenic low MWt toxins
- Injection of up to 15g of foreign (equine) antibodies

In different parts of the world, medically significant venomous snakes can be different, the venom profiles can be also different. In India alone, there are more than 60 species of venomous snakes. It is impossible to have antivenoms at the species level. So, it is important to look for something that unifies the venoms and makes it possible to make treatment for all species.

Understanding Venom Proteomics

Snake venoms are mixtures of different protein families, and each of these families contains many different toxins or toxin isoforms. Medically significant venomous snakes are almost entirely front-fanged and are classified into three families:

1. Atractaspidae (69 species)
2. Elspidae (360 species)
3. Viperidae (Vipers, 340 species)
   a. Viperinae (True vipers, 101 species)
   b. Crotalinae (Pit vipers, 239 species)

The venom glands of advanced snakes are homologous, and current evidence suggests that the three families of front-fanged snakes evolved from non-front-fanged venomous snakes. As snake venom glands are homologous, it would be expected that some toxin families would be ubiquitous across the three front-fanged snake families. However, the ancestral venom proteome has since diversified among different snake families due to the influence of genetic mutations, genetic drift, and natural selection differentially molding the venom of each species to confer optimal prey specific toxicity.

The paper does proteome compositional studies of 132 snake species: 42 from 360 (12%) Elapidae (elapids), 20 from 101 (20%) Viperinae (true vipers), 65 from 239 (27%) Crotalinae (pit vipers), and five species of non-front-fanged snakes.

Approximately 90% of their total venom composition consisted of

1. 8 protein families for elapids
2. 11 protein families for viperines
3. 10 protein families for crotalines

There were four dominant protein families:

1. Phospholipase A2s (PLA2s) (the most common across all front-fanged snakes)
2. Metalloproteases
3. Serine proteases
4. Three-finger toxins (3FTx)

There were six secondary protein families:

1. Cysteine-rich secretory proteins
2. L-amino acid oxidases
3. Kunitz peptides
4. C-type lectins / snaclecs
5. Disintegrins
6. Natriuretic peptides

Elapid venoms contained mostly three-finger toxins (3FTx) and phospholipase A2s (PLA2s) and viper venoms metalloproteases, phospholipase A2s and serine proteases. Although 63 protein families were identified, more than half were present in <5% of snake species studied and always in low abundance. The importance of these minor component proteins remains unknown.

The major difference between elapid and viper venoms was the presence of 3FTx in elapid venoms and the virtual absence of 3FTx in viper venoms. Elapid venoms were also less diverse in the range or number of protein families, largely consisting of only PLA2 and 3FTx, although different groups were dominated by one or the other. Elapid venoms were more variable in the amount of different protein families compared to viper venoms.

Despite this diversity, with only a few exceptions, more than 90% of elapid and viper venoms were composed of just ten protein families. Based on their compositional importance and ubiquity, these 59 protein families were classified into five groups.

1. Dominant protein families (four families): PLA2, SVMP, SVSP and 3FTx
2. Secondary protein families (six families that were commonly present, but in much smaller amounts than the dominant families): KUN, CRiSP, LAAO, CTL, DIS, and NP

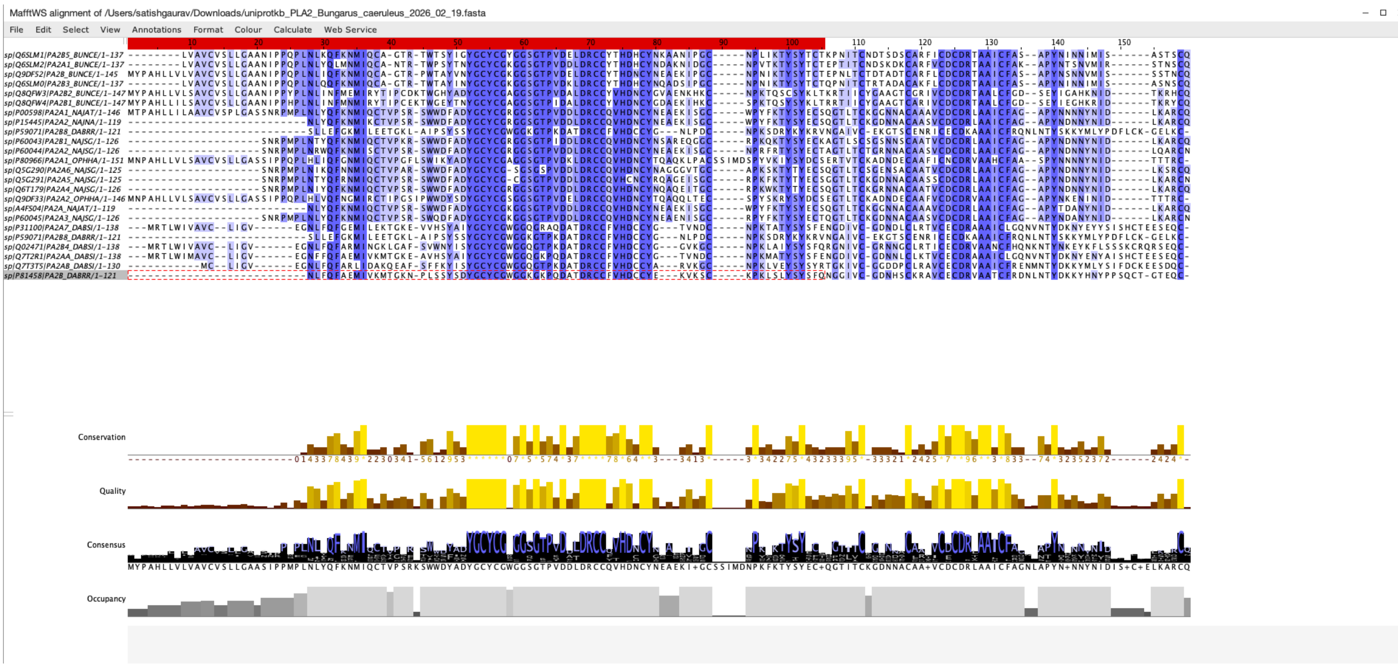
3. Minor protein families (nine families): acetylcholinesterase, hyaluronidase, 5′ nucleotidase, phosphodiesterase, phospholipase B, nerve growth factor, vascular endothelial growth factor, vespryn/ohanin and snake venom metalloprotease inhibitor
4. Rare protein families: 36 families
5. Unique protein families (four families): defensins, waglerin, maticotoxin and cystatins. These families make up to 38% of the whole venom of a single species, but are classified separately as each is present in only one genus

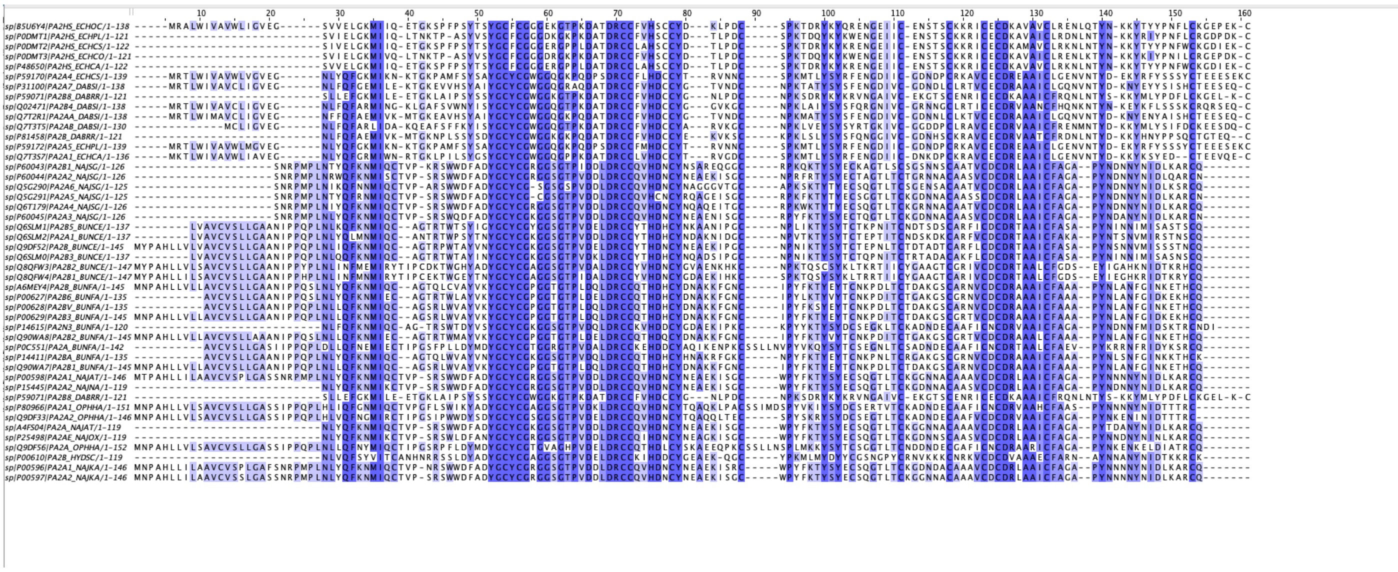
Proteomics of Venomous Snakes in India

The following list shows venomous snakes of interest in India. The paper also highlights that neutralisation efficacy of current polyvalent antivenom on non-big four venomous snakes is either poor or not effective at all. It advocates for the urgent shift to inclusive antivenom strategies that integrate local venom profiles to enhance treatment effectiveness. However, given the high cost of maintaining the animal based animal facility, it is going to be a huge challenge. Also, even though polyvalent antivenom is important in saving lives, its efficacy remains a challenge.

From a proteomics point of view, it is possible to understand the venom profiles and target the important proteins and peptides which make up high portions of the venom dose. Targeted neutralizing proteins can help neutralize the venom proteins more effectively. However, it is important to understand if apart from the venom composition, the protein itself is different, in that case, it will be hard for a single protein to target all venoms.

We tried to find the structural similarity/dissimarlity of the dominant groups proteins in the all venomous snake of India. For now, we focused on the two dominant groups of proteins 3FTXs and PLA2s. We performed multiple sequence alignment of possible protein structures.





We see that venom proteomics varies in all species, however, we see that, few regions of the proteins which are conserved in the proteins. The conserved region is important for preserving protein structure and therefore its function. Evolution is important for nature and it is influenced by the local climate. However, the process is slow, too fast evolution can make any species go extinct. These conserved regions hold the key for universal antivenom therapeutics. Which will be discussed later.

Sl No.	Species (scientific name)	Scientific name	Family	Neutralization by current polyvalent ASV	Geographic distribution	Wiki Link

Big four venomous snakes of India						
1	Indian Cobra	Naja naja	Elapidae			https://en.wikipedia.org/wiki/Indian_cobra
2	Common krait	Bungarus caeruleus	Elapidae			https://en.wikipedia.org/wiki/Common_krait
3	Russell's viper	Daboia russelii	Viperidae			https://en.wikipedia.org/wiki/Russell's_viper
4	Saw-scaled viper	Echis carinatus	Viperidae			https://en.wikipedia.org/wiki/Echis_carinatus
Non-big four venomous snakes of India						
5	Sind krait	Bungarus sindanus	Elapidae	Poorly neutralized	Northwestern India: Rajasthan, Punjab, Gujarat	https://en.wikipedia.org/wiki/Bungarus_sindanus
6	Hump-nosed pit viper	Hypnale hypnale	Viperidae	Not neutralized	Southwestern India: Western Ghats, Kerala, Karnataka and Konkan	https://en.wikipedia.org/wiki/Hypnale_hypnale
7	Salazar's pit viper	Trimeresurus salazar	Viperidae	Not neutralized	Northeastern India: Arunachal Pradesh	https://en.wikipedia.org/wiki/Trimeresurus_salazar
8	Mountain pit viper	Ovophis monticola	Viperidae	Not neutralized	Himalayas, northeast India	https://en.wikipedia.org/wiki/Ovophis_monticola
9	Hook-nosed sea snake	Enhydrina schistosa	Elapidae	Not neutralized	Indian Ocean coasts, Bay of Bengal, Arabian Sea	https://en.wikipedia.org/wiki/Hydrophis_schistosus
10	Andaman's cobra	Naja sagittifera	Elapidae	Not neutralized	Andaman Islands	https://en.wikipedia.org/wiki/Andaman_cobra
11	Banded krait	Bungarus fasciatus	Elapidae	Partially neutralized	Eastern and northeastern India	https://en.wikipedia.org/wiki/Banded_krait
12	Himalayan pit viper	Gloydius himalayanus	Viperidae	Not neutralized	Western Himalayas	https://en.wikipedia.org/wiki/Gloydius_himalayanus
13	Annulated sea snake	Hydrophis cyanocinctus	Elapidae	Not neutralized	Bay of Bengal, Andaman Sea	https://en.wikipedia.org/wiki/Hydrophis_cyanocinctus
14	Greater	Bungarus	Elapidae	Not	Eastern	https://en.wikipedia.

	black krait	niger		neutralized	Himalayas, northeast India	org/wiki/Greater_black_krait
15	Large-scaled pit viper	Craspedocephalus macrolepis	Viperidae	Not neutralized	Southern Western Ghats, India	https://en.wikipedia.org/wiki/Craspedocephalus_macrolepis
16	Central Asian cobra	Naja oxiana	Elapidae	Not neutralized	Northwestern India: Jammu and Kashmir	https://en.wikipedia.org/wiki/Caspian_cobra
17	Lesser black krait	Bungarus lividus	Elapidae	Not neutralized	Northeastern India	https://en.wikipedia.org/wiki/Lesser_black_krait
18	Malabar pit viper	Craspedocephalus travancoricus	Viperidae	Not neutralized	Western Ghats, India	https://en.wikipedia.org/wiki/Craspedocephalus_malabaricus
19	Schouker's saw-scaled viper	Echis carinatus sochureki	Viperidae	Partially neutralized	Northwestern India	https://en.wikipedia.org/wiki/Echis_carinatus_sochureki
20	Red-necked keelback	Rhabdophis subminiatus	Colubridae	Not neutralized	Northeast India	https://en.wikipedia.org/wiki/Rhabdophis_subminiatus
21	King cobra	Ophiophagus hannah	Elapidae	Negligible neutralization	Southwest and northeast India	https://en.wikipedia.org/wiki/King_cobra
22	Northern large-scaled pit viper	Craspedocephalus macrolepis	Viperidae	Not neutralized	Southern Western Ghats, India	https://en.wikipedia.org/wiki/Craspedocephalus_macrolepis
23	Short sea snake	Hydrophis curtus	Elapidae	Not neutralized	Indian coastal waters	https://en.wikipedia.org/wiki/Hydrophis_curtus
24	Indian monocled cobra	Naja kaouthia	Elapidae	Poorly neutralized	Northeast India, especially in Odisha, Assam, West Bengal and parts of the northern plains	https://en.wikipedia.org/wiki/Monocled_cobra