

Venomics: targeted drug discovery in animal venoms

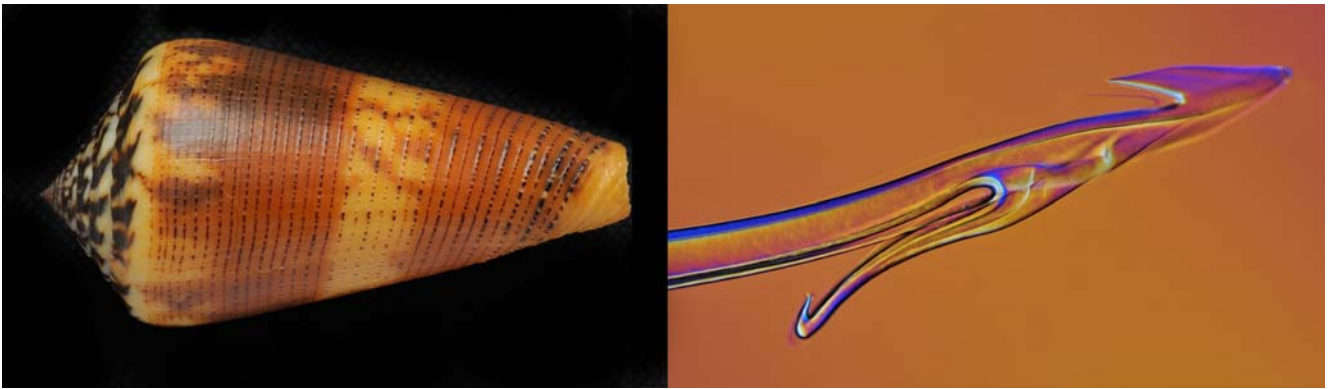
While venomous animals are often considered as a threat that kills, venom components can also serve mankind and cure life-threatening disorders. Animal venoms are complex biofluids made of hundreds of bioactive peptides and proteins that have been evolved by Nature to generate highly selective, potent and stable biomolecules. These ready-made biologics represent a goldmine for the discovery of leads to target ion channels, receptors, enzymes, cells or living organisms. Indeed, there are already five venom-derived peptide drugs on the market (see Table below), and many more are currently undergoing pre-clinical or clinical development to treat for example cancer, pain, multiple sclerosis, stroke, allergies, diabetes or microbial infections.

Company	Drug	Molecule	Species	Target	Disease
Amylin & Eli Lilly	Exenatide (Byetta)	Peptide (natural)	<i>Heloderma suspectum</i> (Gila monster)	GLP-1 receptor	Type-2 diabetes
Bristol-Myers-Squibb	Capoten (Captopril)	Peptide (natural)	<i>Bothrops jararaca</i> (Brazilian lancehead)	Angiotensin converting enzyme	Hypertension
COR Therapeutics	Integrilin (Eptifibatid)	Cyclic peptide	<i>Sistrurus miliaris barbouri</i> (Southeastern pygmy rattlesnake)	Platelet aggregation	Ischemic stroke
Elan Corporation	Ziconotide (Prialt)	Mini-protein (natural)	<i>Conus magus</i> (Magician's cone)	N-type voltage-gated Ca ²⁺ channel	Severe chronic pain
Medicure Pharma & Merck	Tirofiban (Aggrastat)	Peptidomimetic	<i>Echis carinatus</i> (Saw-scaled viper)	Platelet aggregation	Angina & infarction

The venomous function

Venomous animals are represented by nearly 200'000 described species covering the whole animal kingdom and broadly spread towards the planet. They can be found day and night on earth (such as snakes, spiders, scorpions, ants, leeches, ticks, toads and frogs), in the air (bees, wasps, mosquitoes) and underwater (cone snails, sea anemones, corals, jellyfishes and fishes) in almost all climatic areas. They are characterised by a venom gland (secretory cells derived from the salivary glands some 200 million years ago) coupled to a delivery system (fangs, stingers, harpoons or nematocysts for example). This fascinating machinery has been optimised by Nature through million years of evolution to allow these animals to capture and digest their preys, as well as to protect themselves from predators and other enemies.

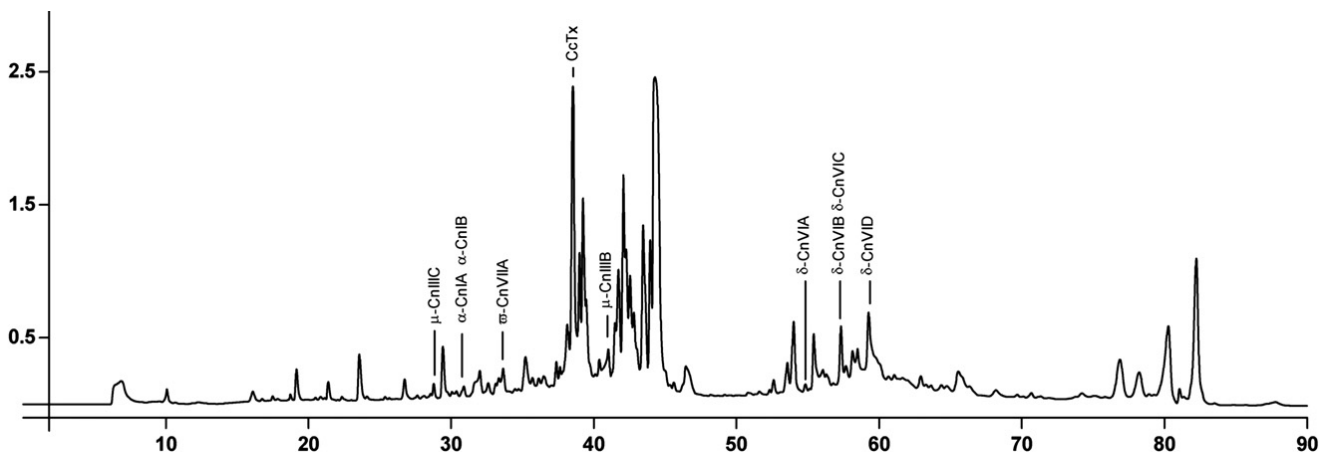
Animal venoms are complex biofluids, each made of hundreds of distinct bioactive molecules, mainly peptides, mini-proteins and enzymes. While the cocktail is able to cause lots of damage, each individual venom component can be seen as a highly selective, potent and stable bioactive. These ready-made biologics represent a goldmine for the discovery of leads targeting ion channels, receptors, transporters, enzymes, cells or living organisms.



*Cone snails use highly sophisticated harpoons (the one represented here is a few millimetres long like a micro hypodermic needle) to inject the venom in their preys to paralyse them within less than a second. Illustrated here is *Conus magus* (magician's cone), a fish-hunter at the origin of Prialt.*

Venom peptides and mini-proteins

The venom of only a few hundred species has been studied to date and only a few thousand components have been characterised so far. Venom components are typically classified in families based on their structural scaffolds and/or pharmacological target. Besides small organic molecules, linear peptides and large proteins, the so-called mini-proteins are doubtless amongst the most fascinating and promising venom bioactives. Mini-proteins are typically ranging from 10-70 amino acids and folded by one to five disulfide bonds. They are extremely stable (resistant to proteases, thus also poorly immunogenic) and soluble (dried venom representing typically 30-50% of the wet weight), they offer high selectivity (towards ion channel sub-types for example) and potency (typically in the low nM or pM range) and most of them can be produced by chemical synthesis efficiently and in a cost effective manner.



*The mass spectrometry of pre-fractionated *Conus consors* venom revealed more than 1'700 molecules, mostly peptides and mini-proteins.*



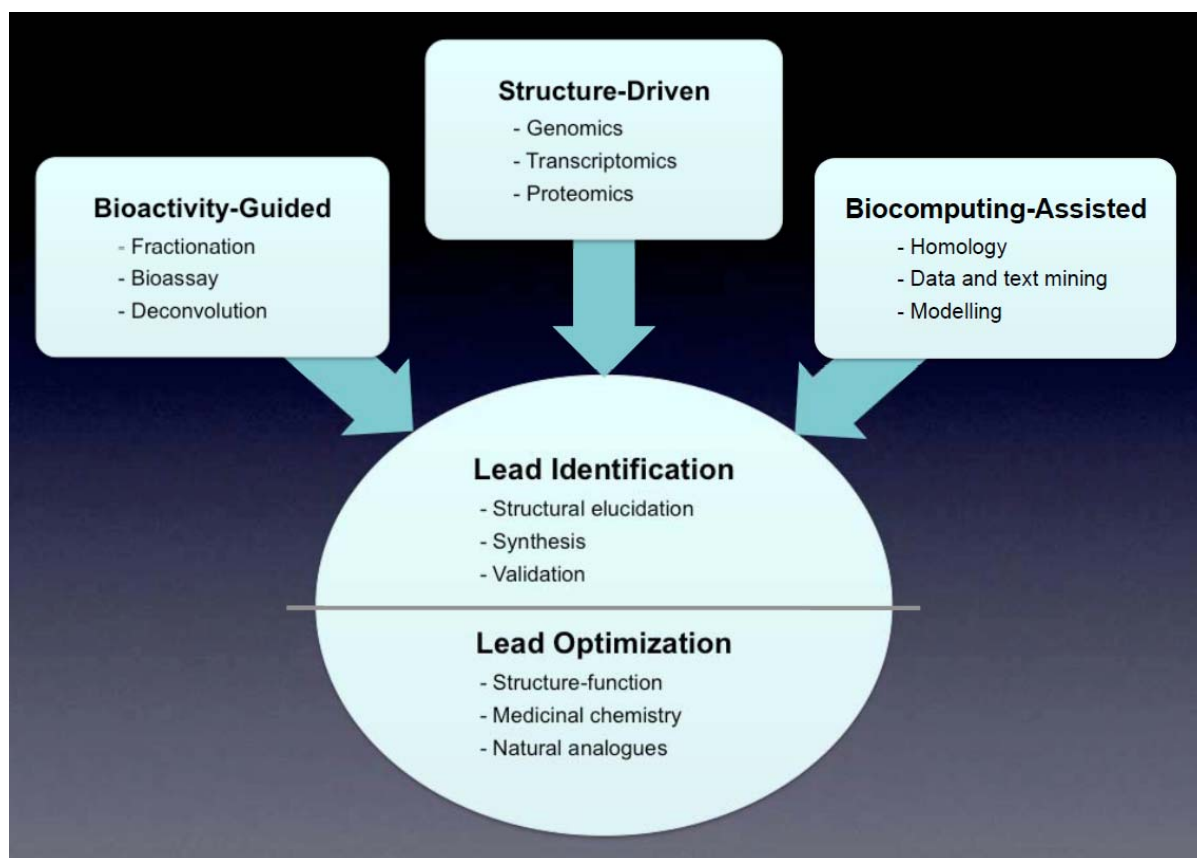
A few typical 3D-folds found in animal venoms. Changing 2-3 amino acids can deeply impact on selectivity or potency of the mini-protein.

Bioactivity-guided, structure-driven and biocomputing-assisted lead discovery

Usually, a bioassay is the first step taken in the quest for new biomolecules, followed by the isolation and characterisation of the native bioactive substance from natural libraries. This bioactivity-guided strategy is time consuming and requires large amounts of material. Nowadays, state-of-the-art proteomic, transcriptomic and post-genomic technologies coupled to bioinformatics can generate an abundance of valuable data not only in a very short period of time, but more importantly using much smaller sample amounts.

For a given target, the venom samples are judiciously selected from our *Melusine* collection to generate better hit-rates. Hits arising from bioassays are submitted to peptidomic analyses. The deconvolution process to identify the bioactives is facilitated by the use of proprietary databases and bioinformatic tools to match mass spectra against public and proprietary protein, EST and genome databases. Our strategy offers unprecedented hit and hit-to-lead rates. It further allows identifying natural analogues of hits, instrumental information for structure-activity studies and lead optimisation.

Atheris has pioneered structure-driven and biocomputing-assisted *Venomics* drug discovery strategies. The venom itself is pre-fractionated to generate a “natural library” of compounds, and hits arising from bioassays are submitted to peptidomic analyses. The deconvolution process to identify the bioactive in fractions of the natural library is facilitated by the use of proprietary bioinformatic tools to match mass spectra against public and proprietary protein, EST and genome databases. Our strategy further allows identifying natural analogues of hits, instrumental information for structure-activity studies and lead optimisation. Novelty, relevance and drugability of hits are similarly evaluated at an early stage for lead selection.



Schematic view of our integrated platform for lead discovery. The concerted efforts from bioactivity tests, primary structural data analysis and biocomputing knowledge allows for a rapid hit identification, thus efficiently enlarging the discovery pipeline for new research tools and potential lead compounds.

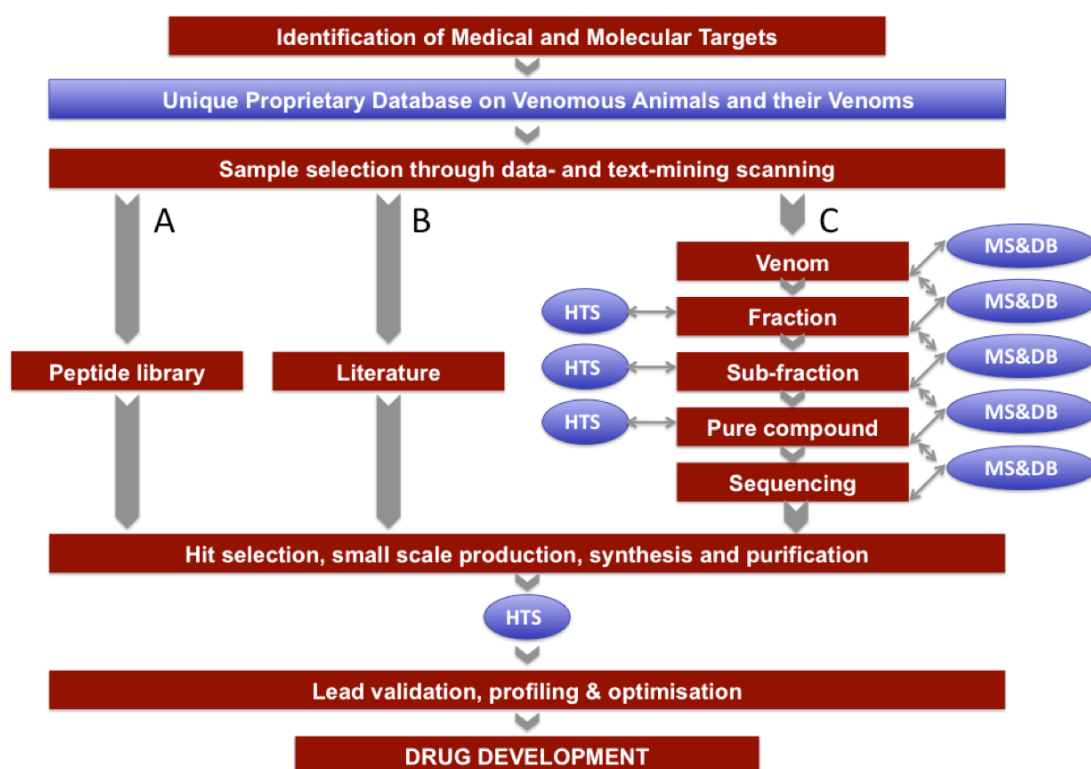
Bioinformatics: proprietary knowledge-based tools

Venoms, our proprietary platform with databases and bioinformatic tools contains a wealth of information over more than 2'000 described venomous animals, 15'000 toxins and antimicrobial peptides, files describing clinical symptoms after envenomation and over 300'000 bibliographical references in the field of venomous animals and their venoms also covering patents.

Our methodology involves an iterative process between *in silico* and *in vitro* strategies:

- Bioinformatics – data- and text-mining as well as statistics
- State-of-the-art separation and sequencing techniques
- Mass spectrometry
- Peptide sequencing and peptide synthesis

These state-of-the-art platforms allow us to undertake extremely focused discovery programmes, in a cost- and time-effective manner. Using our *Melusine* libraries of bioactive peptides and proteins, and in collaboration with industrial and academic partners, we carry out R&D efforts across the discovery chain, spanning from the identification of drug candidates to the selection of lead candidates.



Our approach is an iterative process in which we first evaluate the physiological target and select venoms from the Melusine collection, synthetic peptides from our library and sequences from the literature in a targeted manner for better hit-rates.

There is an ongoing evaluation by mass spectrometry (MS) and matching against our databases (DB) of hits generated in the frame of high throughput screening assays (HTS) throughout the whole deconvolution process.

This speeds up the identification process and also allows discarding hits that do not match given criteria or false positives at very early stages. Once a hit is identified, we have dedicated analytical and biocomputing tools to screen for natural analogues in the same venom or in that of related species that are sometimes not available in sufficient amounts to conduct bioassays, which speeds-up the lead optimisation process and gives clues about which structural features Nature has modified to modulate the activity.

research & development

Atheris
Laboratories

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