Editorial
Special Focus Issue: Venomics

Future Medicinal Chemistry

The European FP7 Venomics Project

"...90% of the biodiversity represented by venomous animals is less than 1 cm in size and has never been explored due to lack of sufficient material."

Keywords: drug discovery • proteomics • toxins • transcriptomics • venoms

The Foundation of the Venomics Project was based on the observation that animal venoms constitute a natural library of several millions of molecules which is largely unexplored and can be exploited as a source of potential drugs. Already six peptide drugs actually in the market are derived from venoms, tens are in clinical development and hundreds of patents have been filed in this field highlighting the therapeutic potential of this natural source.

These molecules include proteins with enzymatic activities such as metallo- or serine-proteases, phospholipases A2, L-amino-oxydase and mini-proteins called toxins that are characterized by their small sizes (less than 100 amino acids) and their enrichment in disulfide bonds (from 1 to 5). These toxins have been selected during the evolution process in order to confer to venomous animals the ability to subdue their prey or to defend against predators. In that goal, they selected in their venoms a large diversity of toxins, enzymes and small molecules that can affect drastically physiological systems of the victims such as the CNS or PNS, the cardiovascular system and the hemostatic system.

Toxinologists, who have been interested for 30 years in the study and exploitation of venoms, have developed different strategies over time to identify new toxins with interesting functional properties. Using a low-throughput bioassay-guided approach, toxins often associated with the major part of the venom's toxicity were identified and their modes of action on ion channels, nicotinic receptors and proteins involved in the coagulation cascade, elucidated. By this approach, hundreds of toxins were characterized pharmacologically, structurally and used as pharmacological tools to study the functional role of their molecular targets. Some of them, due to their high stability, specificity and potency, were therapeutically exploited, such as the eptifibatide (Integrilin®) from the pygmy rattlesnake as antiplatelet drugs in the treatment of acute coronary syndromes or the ω-conotoxin MVIIA (Prialt) from the *Conus magus* for severe chronic pain treatment [1,2]. That classical way of studying venoms is of low throughput and adapted only for big animals from which enough venoms can be obtained. But 90% of the biodiversity represented by venomous animals is less than 1 cm in size and has never been explored due to lack of sufficient material. For the last few years, transcriptomes of venom glands from various venomous animals were obtained revealing their high complexity (the presence of up to a thousand peptides) [3–6] and highlighting the interest of exploring this huge diversity more carefully.

The goal of the FP7 VENOMICS project (2012–2015) is to develop an innovative 'omics' strategy adapted to the investigation of a large diversity of venoms from spiders, snakes, scorpions, cone snails, insects, among others, through the development of a high-throughput workflow integrating cutting-edge transcriptomic and proteomic approaches and massive production of toxins. Thus, the objective is to recreate *in vitro* part of the natural library of toxins adapted for high-throughput screening, by appropriate functional tests applied on selected

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therapeutic targets. Such a type of project can only be achieved on a European scale by combining skills and expertise from academic (CEA, Liège University, KU University and Marseille University) and industrial partners (VenomeTech, Sistemas Genómicos, NZYTech, Zealand Pharma, Vitamib).

The VENOMICS project is separated into two parts, a research and development phase dedicated to the building and optimization of cutting-edge technologies in transcriptomic, proteomic and production of reticulated toxins and a demonstration phase focused on the generation of a large sequence database and manufacturing a peptide bank, ready to be screened.

During the first 2 years, about 100 samples biobank of venoms and venom gland tissues were constituted covering a large diversity of venomous animals. To perform transcriptomic analysis, two technologies, 454 Roche and Illumina, were compared. Illumina has better sensitivity and thanks to a powerful assembling protocol that provides many more reading frames. High-throughput *ab initio* proteomic analysis of venom peptides also represents a real challenge. Two different strategies are developed for venoms containing small toxins (less than 40 residues, such as cones, insects and spiders) and for larger toxins from snakes and scorpions. The sequence tags from proteomics are compared with the transcriptomic sequences to confirm the presence in the venom of the toxins and to identify post-transcriptional modifications (PTM), if any. Data coming from both transcriptomic and proteomic analysis provide a validated sequence database with currently between 100 and 200 sequences per animal. The last step of the VENOMICS project is to manufacture a peptide bank of 10,000 toxins, by two complementary strategies. Solid-phase synthesis has been chosen for peptide of less than 40 residues with PTM while longer ones will be produced by recombinant expression in *Escherichia coli*. Both strategies are in terminal phase of validation.

In conclusion, VENOMICS can be seen as a project intending a dual rupture in toxinology. The first one concerns the development of cutting-edge technologies adapted to any venomous animals whatever their sizes and focused on the *in vitro* reproduction of venoms in a synthetic bank compatible with high-throughput screening. The second breakthrough would be for venoms to no longer be considered as a mixture of highly poisonous toxins for the preys, historically associated with neurotoxic activities or affecting the blood coagulation process, but as a natural source of biologically active peptides. These peptides may be used as invaluable tools for probing various physiological processes or serve as leads for drug discovery in relation to human health. In that goal, VENOMICS project tends to identify new bio-drugs by screening the synthetic toxin library on specific molecular targets known to be involved in diseases representing unmet therapeutic needs with associated markets.

These objectives are now commonly shared by toxinologists all around the world with the general idea to bypass the slow bioassay-guided fractionation workflow currently used in venom's studies by optimizing the transcriptomic and proteomic analyzes of venoms as well as the toxin's production process.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

No writing assistance was utilized in the production of this manuscript.

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