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PRESENTATION

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Founded in 1995 by Dr Reto Stöcklin, Atheris Laboratories is a family-owned and family-driven biotechnology company specialised in peptide and protein drug discovery, peptidomics, bioinformatics, metabolic studies and analytical services. Atheris uses state-of-the-art methods to offer highly specialised services as contract research organisation or as service provider.

Atheris is a dynamic research-driven company with a strong technology platform and a unique expertise in mass spectrometry, biochemistry, protein engineering, bioanalytical pharmacokinetic and metabolic studies (PK-ADMET). Atheris has accumulated several decades of experience investigating bioactive compounds from a variety of venomous organisms and other natural sources. These lead discovery and lead optimisation programs led to the building of a unique, world-class expertise pertaining to the extraction, isolation, purification, characterisation and synthesis of bioactive peptides.

Atheris pursues a two-pronged development strategy:

- Accelerate the development of its unique **Lead Discovery Platform** through internal efforts, academic and industrial collaborations.
- Consolidate its technology platform and broaden the range of **R&D Services** offered to the Pharmaceutical, Biotechnology, Cosmetic and Food industries, also through contract research.

Using a proprietary library of bioactive peptides and proteins (see www.melusine.com), and in close collaboration with its partners, Atheris carries out R&D efforts across the discovery chain, spanning from the identification of drug candidates to the selection and optimisation of lead compounds.

Atheris aims to contribute to the **discovery of new drug candidates, bioactive ingredients and diagnostic markers**, for the improvement of health care in a broad range of areas such as the Central Nervous, Cardiovascular, and Immune systems.



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, which has already led five drugs to the market (Captopril, Eptifibatide, Exenatide, Prialt and Tirofiban).

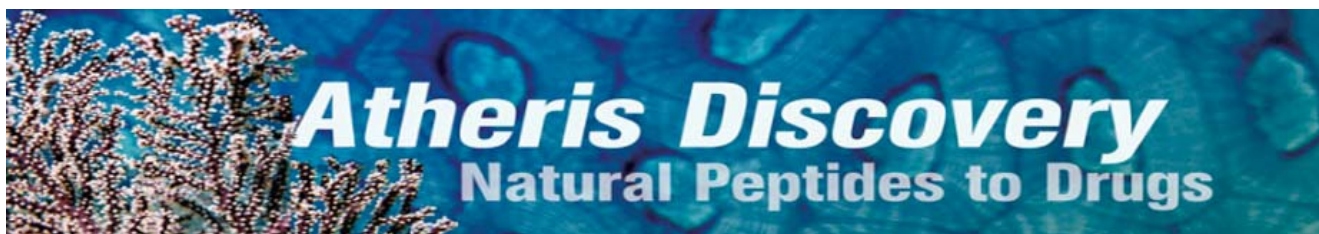
A lead discovery platform that delivers - Usually, a bioassay is the first step taken in the quest for new biomolecules, followed by the isolation and characterisation of the native bioactive substance. This bioactivity-guided strategy is time consuming and requires large amounts of material. Atheris has pioneered structure-driven and biocomputing-assisted **Venomics** drug discovery strategies. State-of-the-art proteomic, transcriptomic and post-genomic technologies coupled to bioinformatics can generate an abundance of valuable data in a very short period of time, at reduced costs and using much less sample.

In close collaboration with its partners, Atheris carries out R&D efforts across the discovery chain, spanning from the identification of drug candidates to the selection and optimisation of lead compounds. Our proprietary platform is based on our long-term expertise coupled to a three-pronged strategy:

- **Melusine:** Unique collections of pre-fractionated venoms, ready-made for HTS
- **Neptilus:** A state-of-the art peptidomics platform optimised for hit deconvolution
- **ToxEnter:** Proprietary databases on venoms with dedicated biocomputing tools

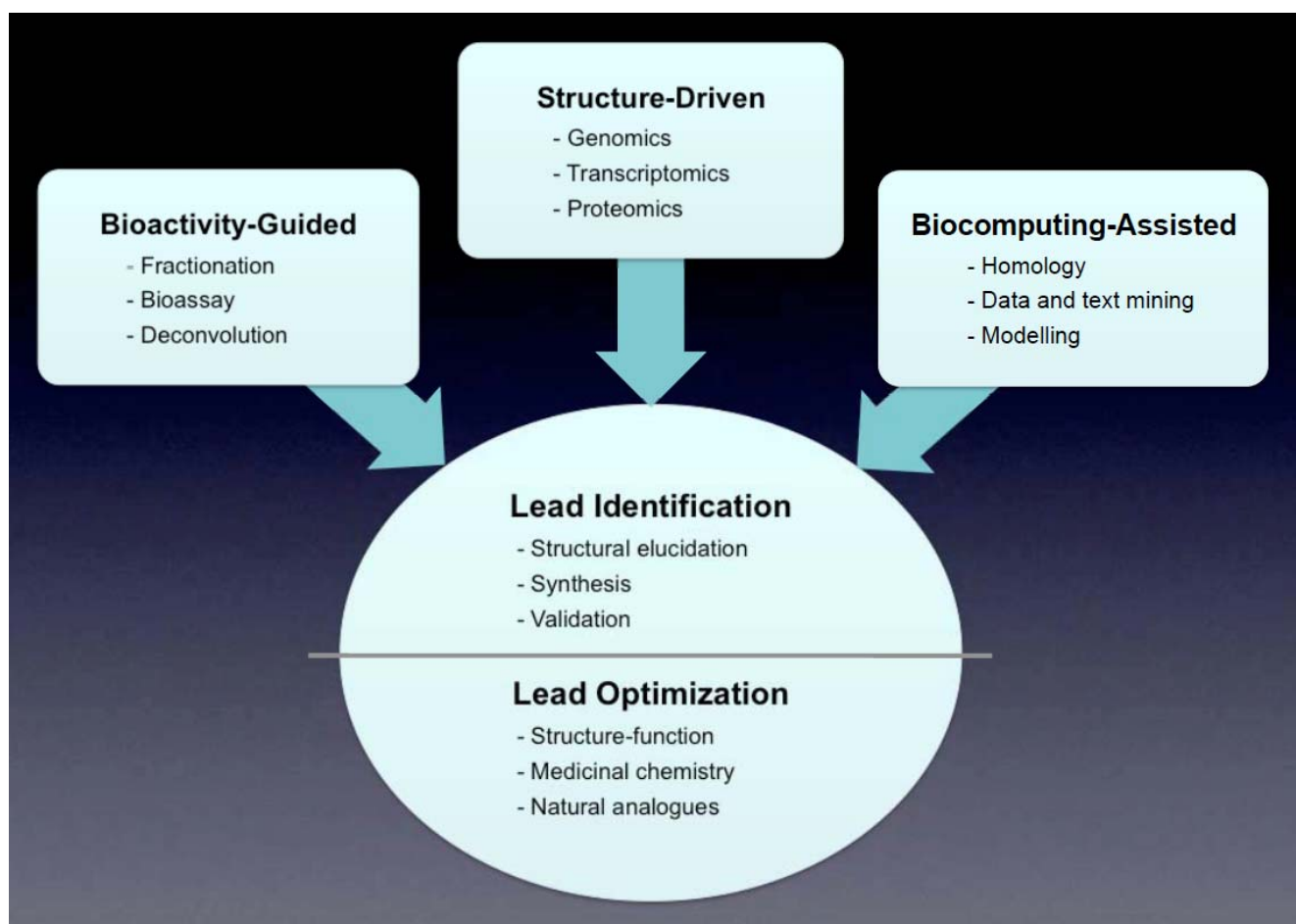
Melusine – unexplored libraries of peptides & proteins for lead discovery

For a given target, the venom samples are judiciously selected from our Melusine collection made of 600 pre-fractionated venoms (48'000 fractions, >300'000 compounds, mostly bioactives) 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.



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.

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.

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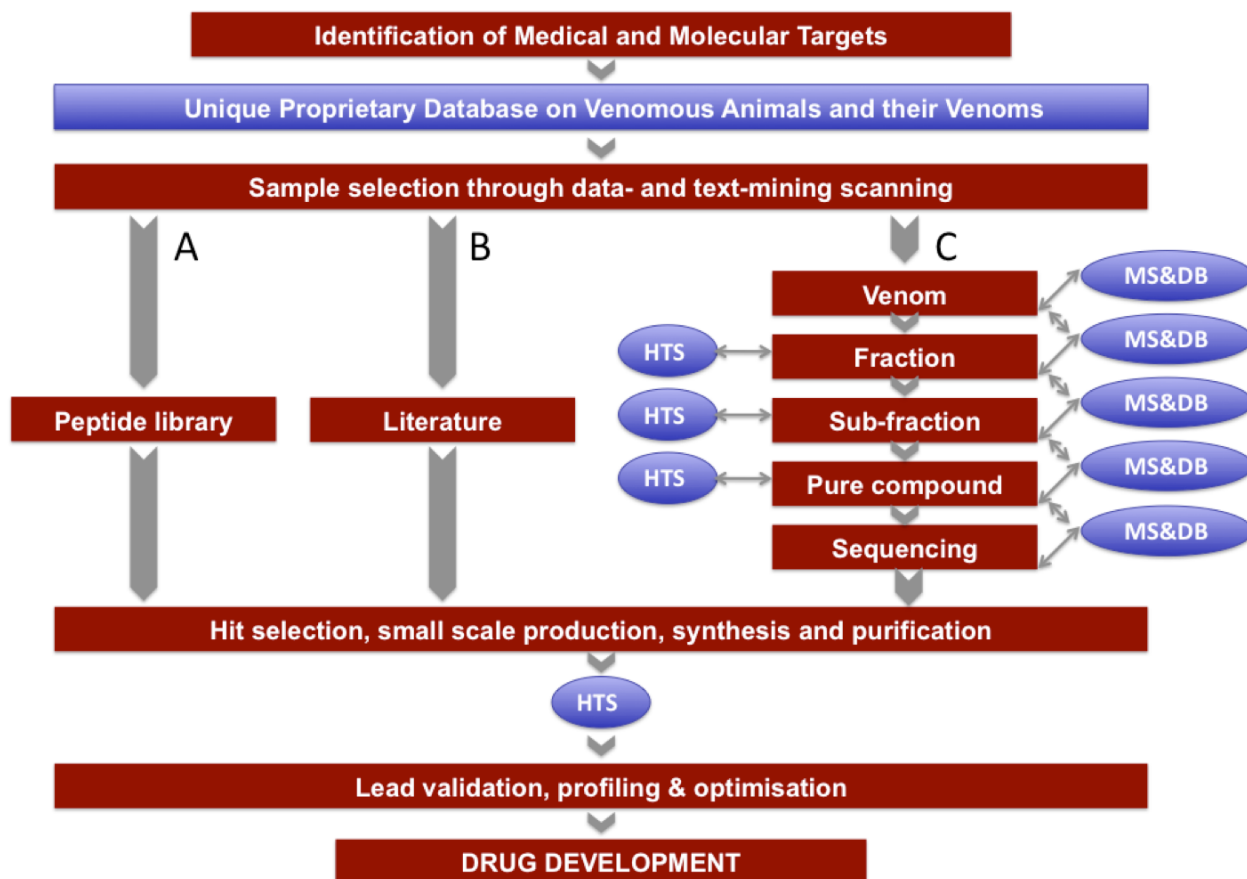
Bioinformatics

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 also dedicated analytical and biocomputing tools to screen for natural analogues in the same venom or in that of related species that are sometimes 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.

Atheris Analytical

Peptides, Proteins and Small Molecules

At Atheris labs we use state-of-the-art separation and analytical methods to offer highly specialised services to the life science industry. Our team of specialists accumulates several decades of experience of investigations in the following areas:

- **Mass Spectrometry, Peptidomics and Proteomics** : Electrospray MS in positive or negative ionisation mode / On-line LC-ES-MS, from nano-bore to preparative scale / MALDI-TOF-MS and MS/MS in linear or reflectron mode / NanoLC-MS/MS structural investigation, tandem mass spectrometry / Protein characterisation, *de novo* sequencing.
- **Protein Chemistry** : Separation and protein purification using LC and HPLC / Edman sequencing / Disulfide bridges / Post-Translational Modifications.
- **Protein Engineering** : Synthesis / Purification / Activity control / Stable isotope labelling / Amino acids modifications / Profiling.
- **Metabolic Studies** : Atheris addresses the crucial issues of Pharmacokinetics and Pharmacodynamics of polypeptides using an attractive method known as IDA or Isotope Dilution Assay. It was developed successfully for quantification of insulin and other peptides in blood samples at pM levels. IDA's inherent characteristics are making it the "gold standard" method for clinical work.

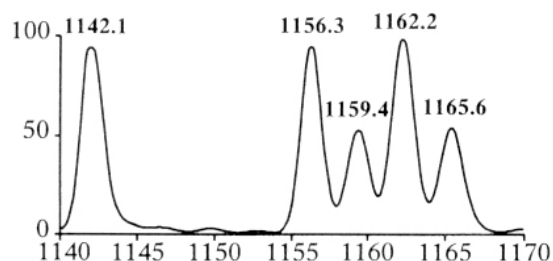
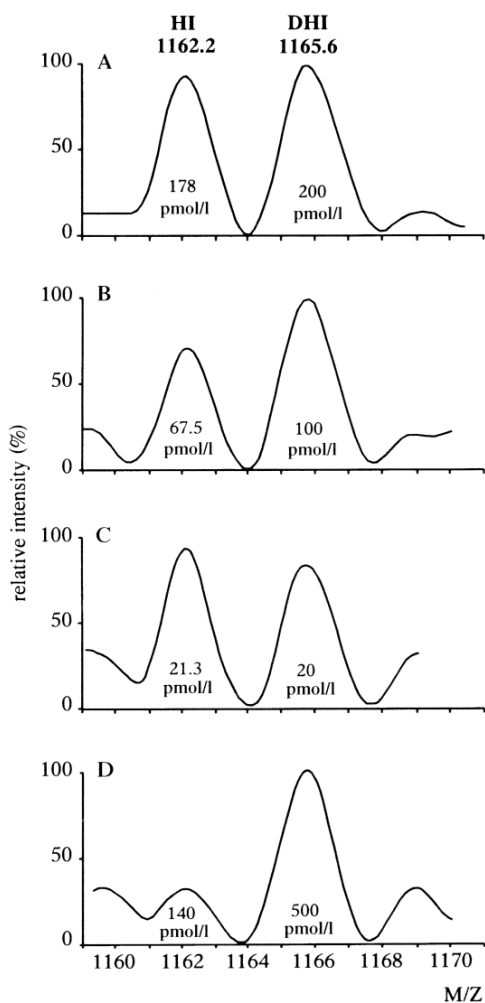
IDA - Isotope Dilution Assay

We have extensive experience using HPLC mass spectrometry (LC-MS) to quantify minuscule concentrations of peptides and proteins in biological fluids and tissues down to basal pM concentrations for PK/PD or ADME studies (absorption, distribution, metabolism and excretion). Highly sophisticated LC-MS and LC-MS/MS methods for investigations related to insulin, proinsulin, C-peptide, NPY or LHRH analogues for example have thus been developed successfully. They allow fast, sensitive and precise measurements of the target compound and its metabolites.

Studies of protein metabolism have so far relied heavily on the use of radioactive tracers or on immunoassays. However, both methods suffer from inherent drawbacks: on the one side a certain aversion to use radioactive labels or difficulties to produce specific antibodies and, on the other side, an indirect detection and cross-reactions often leading to ambiguous or incomplete results. In many cases, Atheris likes to address the crucial issues of *in vivo* metabolic studies of polypeptides using analogues of the target compound labelled with stable isotopes. These non-radioactive analogues have different molecular masses and can thus specifically be followed by mass spectrometry independently of the native unlabelled form of the drug of interest. This drastically facilitates tissue distribution studies and the identification of metabolites of the injected peptide drug.

Dr Reto Stöcklin pioneered the use of stable isotope for *in vivo* PK-PD studies by Isotope Dilution Assay (IDA) for the quantification of insulin C-peptide and proinsulin in blood samples down to low pM levels (a few pg/mL). IDA allows the most accurate and sensitive detection of proteins or peptides by mass spectrometry. In addition, it enables a precise quantification on the basis of the relative intensities of the observed signals (principle of isotope dilution, see figure below). IDA completely avoids the use of radioactive material and is not susceptible to errors arising from immunological dosages. Furthermore, this method can clearly discriminate endogenous and injected forms, thus allowing for simultaneous *in vivo* quantitative investigations of both endogenous and injected compounds. Similar strategies can be used to detect, identify and characterise degradation fragments.

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Above: A mixture of different analogues of the target compound can be used to generate a specific MS pattern that can be followed through the body. The mass spectrum shown corresponds to a 1:1:0.5:1:0.5 mixture of desAlaB30 insulin (DAI, m/z 1142.1); porcine insulin (PI, m/z 1156.3); octadeutero-PheB1-octadeutero-ValB2 porcine insulin (DPI, m/z 1156.3); human insulin (HI, m/z 1162.2); octadeutero-PheB1-octadeutero-ValB2 human insulin (DHI, m/z 1165.6).

Left: Principle of Isotope Dilution Assay (IDA): examples of mass spectra of clinical samples in physiological pM concentrations. The mass spectra for the quantification of human insulin (HI, on the left hand side) were obtained after extraction from clinical serum samples using a known amount of the hexadecadeuterated analogue as internal standard (DHI, on the right hand side).

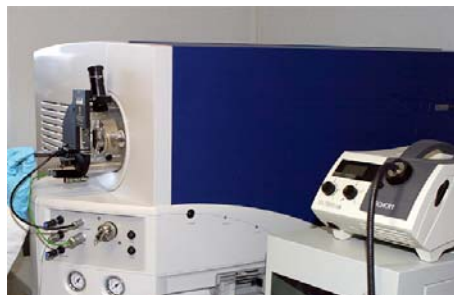
The ionized species carry five protons (H) and so appear at m/z (mass-to-charge) values given by $(M_r + 5H)/5$, where M_r is the relative molecular mass. HI ($M_r = 5807.6$ Da) for example is responsible for the signal at a mass-to-charge ratio of 1162.2.

IDA method development and validation

An Isotope Dilution Assay can thus be developed specifically for a given peptide or protein for its detection and quantitative studies in animal or human blood plasma, possibly also in other body fluids or solid tissues. The methodology will need to be adapted to achieve an acceptable limit of detection (LOD) and lower limit of quantification (LLOQ) in biological samples. Analytical and data treatment processing time will also need to be optimised. Complete or partial validation of the method (selectivity, accuracy, precision, recovery, calibration/standard curve, stability under various analytical conditions, quality controls, etc.) are in principle performed according to the guidance for industry - bioanalytical method validation. Determinations of the LOD and of the LLOQ for the compound of interest, as well as determination of the linearity of the response in standard plasma samples spiked with known amounts of the target peptide are usually achieved prior to be eventually developed and validated again for each of the other tissues investigated.

In vivo PK-ADME studies

IDA allows to detect the peptide of interest present in the blood stream down to its LOD values, and to quantify it precisely down to its LLOQ values in samples generated by *in vivo* animal or human studies. IDA also allows determining the *in vivo* half-life of a peptide in blood, whatever administration method is used. *In fine*, this approach also allows investigating whether an orally administered peptide penetrates the blood stream across the gut, and if so at what levels at with which kinetic parameters. The use of stable isotope labelled analogues further opens the doors to additional investigation such as metabolite identification and characterisation or biodistribution studies.



Top left:
Bench for sample preparation.

Bottom left:
HPLC (Alliance HT, Waters) coupled to ESI-MS/MS mass spectrometer (Quattro micro, Waters/Micromass).



Top right:
Q-TOF mass spectrometer (Waters/Micromass) equipped with nanoflow electrospray ionisation.

Bottom right:
MALDI-TOF-TOF tandem mass spectrometer (Ultraflex, Bruker Daltonics).

At Atheris, a solid team of experts uses state-of-the-art instruments; our equipment presently includes six different and complementary mass spectrometers and ten chromatographic systems.

Atheris is also involved in the following activities:

Activen S.A. - Ingredients for the cosmetic industry
www.activen.ch

CONCO - The cone snail genome project for health.
www.conco.eu

FunZyme Biotechnologies S.A. - Potent enzymes-based drugs and agents for biotechnology and healthcare.
www.funzyme.com

HiQScreen SàRL - High throughput functional electrophysiology bioassays for drug discovery.
www.hiqscreen.com

Melusine® - Libraries of natural, synthetic and virtual venom bioactives for targeted drug discovery
www.melusine.com

NP2D - Natural Peptides to Drugs International congress in Zermatt.
www.np2d.com

The Toxinomics Foundation - Promotion of the knowledge on animals, plants and micro-organisms producing toxins and related substances for the benefit of mankind and nature.
www.toxinomics.org

Vector LifeSciences S.A. - Tissue-selective cell penetrating peptides for health.
www.vector-lifesciences.com