18th World Congress of the International Society on Toxinology

The Examination Schools & The Sheldonian Theatre
Oxford, United Kingdom

Congress Agenda

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Utkin, Professor Yuri, Russia
van Helden, Dr Dirk, Australia
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Wagstaff, Dr Simon, UK
Williams, Dr David J, Papua New Guinea
Wüster, Dr Wolfgang, UK
Zachariah, Professor Anand, India
Zhang, Professor Yun, PR China

KEYNOTE PLENARY SPEAKERS

Professor David Julius, California (San Francisco), USA
Professor Harry Greene, New York, USA
Professor Baldomero Olivera, Utah, USA
Professor Oliver Dolly, Dublin, Ireland
Professor Dr Juan Calvete, Valencia, Spain
Professor Dr Cesare Montecucco, Padova, Italy
Professor Hagan Bayley, Oxford, UK
Professor Angela Vincent, Oxford, UK
Professor Peter Ratcliffe, Oxford, UK

26-9-2: **Rita C O Collaço,** Size-exclusion chromatography on venom: antivenom binding analysis: a new perspective for $F(ab')_2$ antibody fragments study
26-9-3: **Shyam B Dhawan,** Retrospective review of snakebites treated with new Snake venom Antiserum in rural Maharashtra, India
26-9-4: **Gus A Gross,** The Diamond Hour
26-9-5: **Ajith Venugopalan,** A study to estimate the prevalence of adverse reactions to anti-snake venom therapy in a tertiary care centre in South India
26-14-1: **Summer Xia Han,** Gene regulatory elements and evolution of snake toxins
26-19-1: **Sonia Adi-Bessalem,** Pharmaco-modulation of Gastric Inflammatory Response by Histamine H4 Receptor and Cyclooxygenase 2 Pathway during scorpion envenomation
26-19-2: **Aouatef Ait-Lounis,** TNF-alpha modulates adipose macrophage polarization to M1 phenotype in response to scorpion venom
26-19-3: **Naira Ayvazyan,** Morphological and functional alteration of erythrocyte ghosts caused by vipers venom
26-19-4: **Amina Ladjel-Mendil,** Involvement of signaling pathways in the induced neuropathological disorders by Kaliotoxin
26-19-5: **Ladjel-Mendil Amina,** IL-6 and TNF-α involvement in immuno-inflammatory response and oxidative stress induced by *Androctonus* scorpion venom
26-19-6: **Adriana N Martins,** Exposure of lactating rats to the Tityus bahiensis scorpion venom: Effects on behavioral development, neuronal intactness and cytokine levels.
26-19-7: **Gabriel O Meissner,** First recombinant expression and biological characterization of an ICK toxin from *Loxosceles intermedia* (brown spider)
26-19-8: **Paulo A Melo,** Cytotoxicity of *Apis mellifera* Bee Venom: Pharmacological interventions and treatment
26-19-9: **Selvanayagam Nirthanan,** Delineating a potassium channel blocking peptide segment from spinoxin (αKTx6.13), a Kv1 channel inhibitor from *Heterometrus spinifer* scorpion venom
26-19-10: **Fernanda C. Oliveira,** Investigation of cytotoxicity of *Bothrops atrox* venom and purified LAAO in primary keratinocytes
26-19-11: **Thalita Rocha,** The effect of Dipotassium Glycyrrhizinate to minimize the myonecrosis induced by *Bothrops jararacussu* snake venom and to induce muscle regeneration
26-19-12: **Maria R Sandoval,** Effects of an anti-muscarinic component isolated from *Micrurus lemniscatus* venom on inositol phosphate and learning and memory in rats
26-19-13: **Carlos Chavez- Olortegui,** Determination of toxic activities in *Bothrops spp.* snake venoms using animal-free approachs: Correlation between *in vitro* versus *in vivo* assays
26-19-14: **Hossein Vatanpour,** *Ex-vivo* evaluation of the electrophysiological effects of the crude venom of *Buthotus schach* on rat brain neurons.
26-19-15: **Joshua S Wingerd,** A novel tarantula venom peptide with subtype-dependent pharmacology at voltage-gated sodium channels

Presentation Date: Monday 28th September, The North Schools

28-3-1: **Márcia H Borges,** Global profile of the venom of *Grammostola Iheringi* Brazilian tarantula: searching for biotechnological potential
28-3-2: **Michel Degueldre,** Multi-analytical method to characterise *Naja atra* venom
28-3-3: **Mikael Engmark,** High-throughput epitope identification for snakebite antivenom
28-3-4: **Julián Fernández,** Snake venomics of *Micrurus alleni* and *Micrurus mosquitensis* from Costa Rica: two divergent compositional patterns in New World elapids
8-3-5: **Paul M N Heiss,** Venomic characterization and bioactivity screening of *Vipera anatolica*, *Vipera darevskii* and *Montivipera bulgardaghica*
28-3-6: **Florence Jungo,** VenomZone: a new website to unravel venom complexity
28-3-7: **Tai Kubo,** Utilization of neurotoxin-inspired peptide libraries in *in-vitro* evolution, and its proved pluripotency to target GPCRs, proteases and trophic factors
28-3-8: **Rafael D Melani,** Top-down Venomics: mapping intact proteoforms and protein complexes in king cobra venom.
28-3-9: **Bruno Madio,** Proteomic and transcriptomic investigation of the venom from Australian sea anemones provides insight into venom evolution and ecology
28-3-10: **Gilles Mourier,** VENOMICS project: Production of two and three-Disulfide-Bridges small Toxins
28-3-11: **Carlos Correa-Netto,** Monoclonal-based antivenomics and biological activities revealing conserved neutralizing epitopes across Elapidae family
28-3-12: **Carolina A Nicolau,** *Bothrops jararaca* proteopeptidome: extensive molecular characterization of samples to be assayed by the connectivity map approach
28-3-13: **Davinia Pla Snake,** venomics of the palm-pitvipers *Bothriechis bicolor, B. aurifer and B. thalassimus* from Guatemala
28-3-14: **Loic Quinton,** Diversity of peptide toxins from four *Conus* venoms revealed by combined cutting-edge technologies of proteomics, transcriptomics and bioinformatics
28-3-15: **Ene Siigur,** Vipera lebetina venom nucleases
28-3-16: **Leijiane F de Sousa,** Adaptive advantages of individual variation of *Bothrops atrox* venom from snakes collected at different phytogeographical scenarios in Brazilian Amazon
28-3-17: **Ana F Sequeira,** High-Throughput synthesis and cloning of genes encoding venom peptides: developing a platform for the discovery of novel therapeutic molecules
28-3-18: **Wang-Chou Sung,** High Throughput Disulfide Bond Profiling of Crude Snake Venom Using Mass Spectrometry
28-3-19: **Choo Hock Tan,** Venomics of *Hydrophis schistosus*, the beaked sea snake: a simple toxin arsenal cross-neutralised by two heterologous antivenoms
28-3-20: **Kae Yi Tan,** Geographical variations of Naja kaouthia (monocled cobra) venom from Southeast Asia: a venomic and functional study

Venomic characterization and bioactivity screening of Vipera anatolica, Montivipera bulgardaghica and Vipera darevskii

Paul M. N. Heiss[1], Daniel Petras[1], Bayram Göçmen[2], Ayse Nalbantsoy[3], Mert Karış[2], Mehmet Z. Yıldız[4] and Roderich D. Süßmuth[1]

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[2] Ege University, Zoology Section, Department of Biology, Bornova Izmir, Turkey
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INTRODUCTION

The outback of Turkey offers us the rare venomous viper species *Vipera anatolica*, *Vipera darevskii* and *Montivipera bulgardaghica* from which the first two are cataloged in the "IUCN Red List of Threatened Species" as endangered. The current study is focused on the proteomic characterization of their venom composition, using not only a bottom-up approach via RP-HPLC, SDS-PAGE, tryptic digest and finally a de novo sequencing of CID-MS/MS data, but also a top-down proteomic approach from native and chemically reduced venoms. The mixture of peptides and proteins of various families, of which we identified several protease inhibitors, disintegrins, PLA2s, C-type lectins, SVSPs and SVMPs, makes envenoming by these vipers a serious health issue, which requires treatment with a specific antivenom. Resolving the venom proteome will provide useful information on unique toxin compositions of these unexplored species and will help in the design of future polyvalent antivenoms. On the other hand, some venom compounds show promising pharmacological effects against various cancer cell lines, and thus might act as a new template for drug-design.

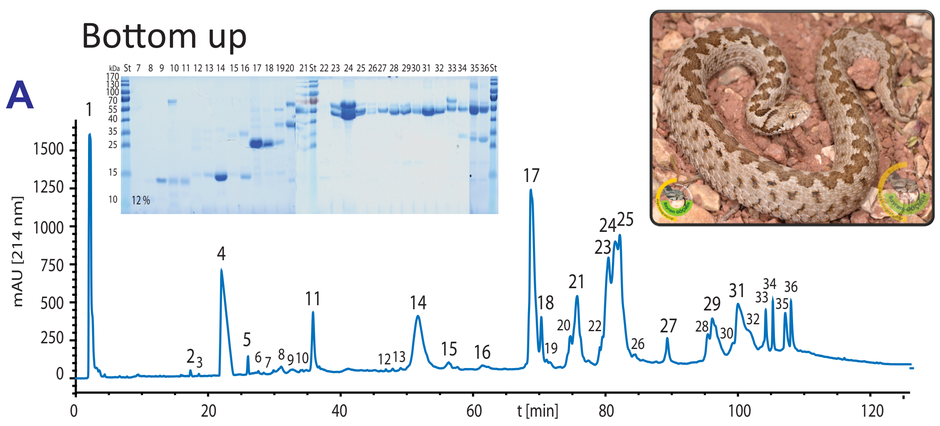
In this poster we show the latest results of our profiling and bioactivity screening studies as well as the technological progress, pushing the limits of our venomic workflow. Future combination of orthogonal approaches such as top-down paired with bottom-up proteomics will hereby provide a more detailed look into a locus resolved composition of snake venoms.

Figure 1. Geographical distribution of *Vipera anatolica* (I), Montivipera Bulgardaghica (II) and Vipera darevskii (III)

I *Vipera anatolica*

RESULTS AND DISCUSSION

Bottom up



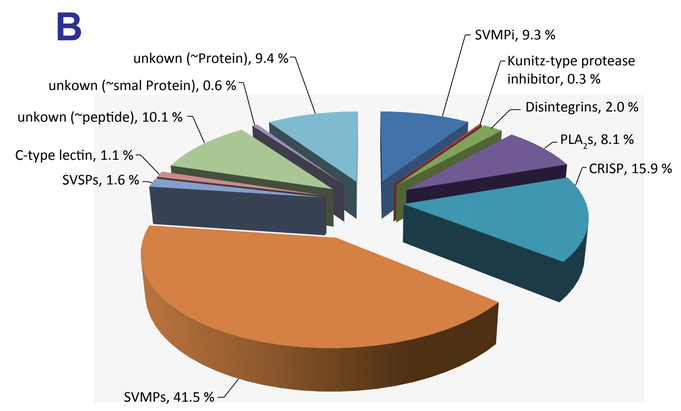
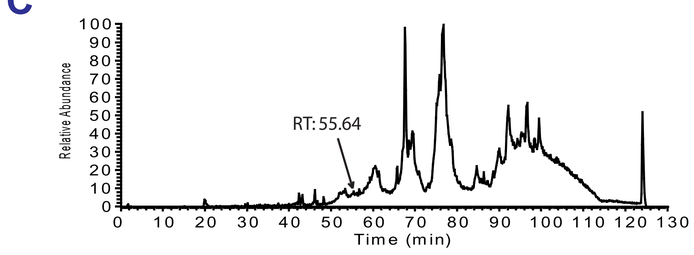
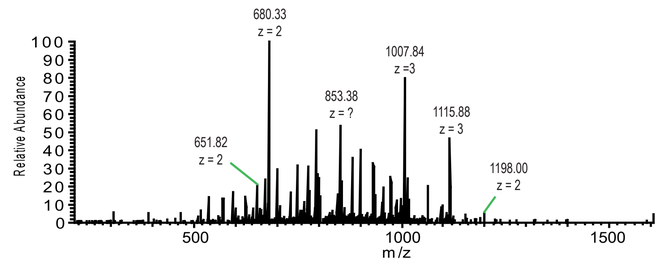


Figure 2. A Representative LC-MS profile and coomassie stained SDS-gels of *Vipera anatolica* venom. B Semi-quantitative venom composition of Vipera anatolica.

Aditional top the usual bottom up approach, consisting of a separation of the compounds via RP/HPLC followed by an in gel digestion of SDS-PAGE (Figure 2) and solving the protein sequence through LC-MS/MS, a new top down approach was performed to identify the peptides and proteins. This method consists of injecting the crude venom directly into HPLC system coupled on an LTQ Orbitrap XL mass spectrometer. The resulting raw data of the LC-ESI-HR-MS/MS experiments where then analyzed using modern bioinformatic tools, such as the deconvolution algorithm of Xcalibur. The resulting *de novo* fragments where matched against a Viperidae database (Figure 3).

Topdown aproach





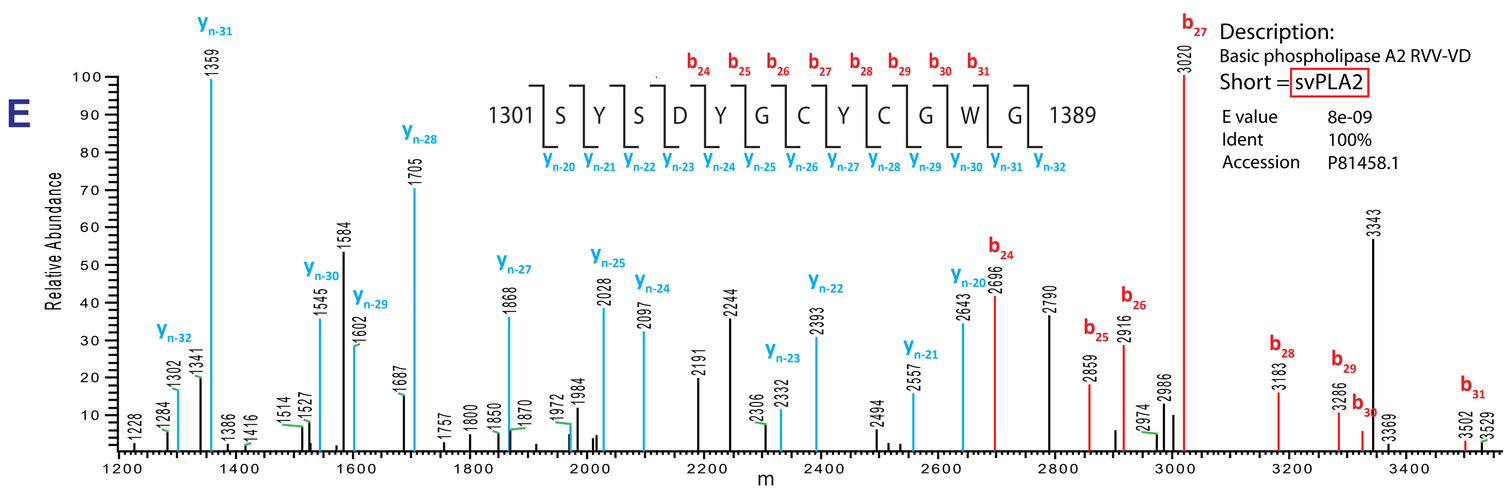
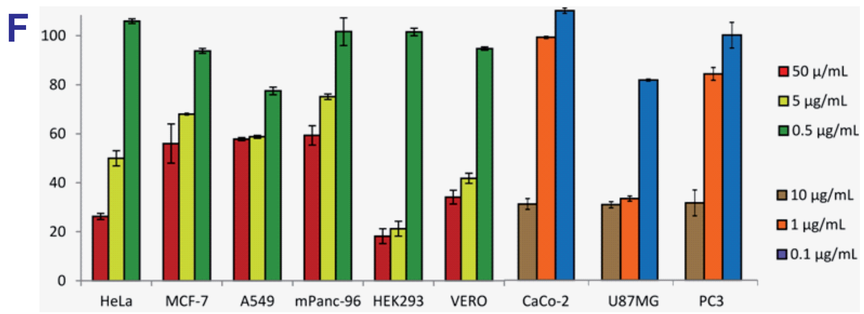


Figure 3. Representative mass spectra of *Vipera anatolica* venom. C shows the Total Ion Chromatogram (TIC). **D** shows the MS/MS spectra of the peptide with the retention time of 55.64. **E** shows a deconvoluted and *de novo* solved spectra of a Basic phospholipase (PLA2).

Bioactivity sceening

Cytotoxicity assays of crude venom against the following cell lines: CACO-2, human colon carcinoma epithelial cells; MCF-7, human breast adenocarcinoma epithelial cells; U87MG, human glioblastoma-astrocytoma epithelial-like cells; PC3, human prostate epithelial cells; HeLa, human cervical epithelial carcinoma cells; MPanc-96, human pancreatic fibroblast cells; A549, human lung epithelial cells; HEK293, human embryonic epithelial kidney cell; Vero, African green monkey fibroblast-like kidney cells were performed. Cytotoxic activity was observed for all three Vipers. The only active fraction (Peak 11), containing a dimeric disintegrin, showed a significant cytotoxic effect on glioblastoma cells with an IC50 value of 0.51±0.04 µg/ml (Figure4).



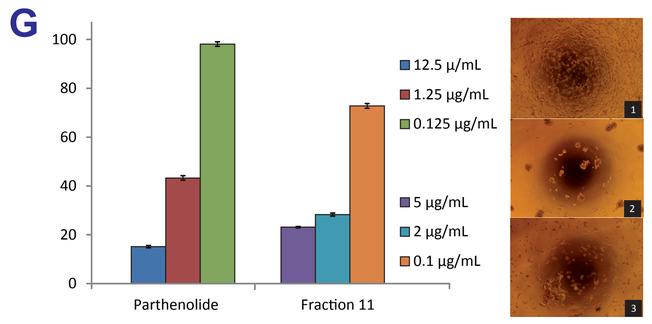


Figure 4. F Viability of cancer and non-cancerous cell lines after crude venom treatment (*V. anatolica*) for 48 h. Cell viability was determined by MTT assay, control was exposed to vehicle only which was taken as 100 % viability. CACO-2, human colon carcinoma epithelial cells; MCF-7, human breast denocarcinoma epithelial cells; U87MG, human glioblastoma-astrocytoma epithelial-like cells; PC3, human prostate epithelial cells; HeLa, human cervical epithelial carcinoma cells; MPanc-96, human pancreatic fibroblast cells; A549, human lung epithelial cells; HEK293, human embryonic epithelial kidney cell; Vero, African green monkey fibroblast-like kidney cells. **G Effect of the isolated disintegrin on U87MG cells.** Cells were treated with fractionated venom (fraction 11) for 48 h at 37°C. 1: untreated, 2: treated with peak 11 2 µg/ml, 3: treated with parthenolide, 1.25 µg/ml.

II *Montivipera bulgardaghica*

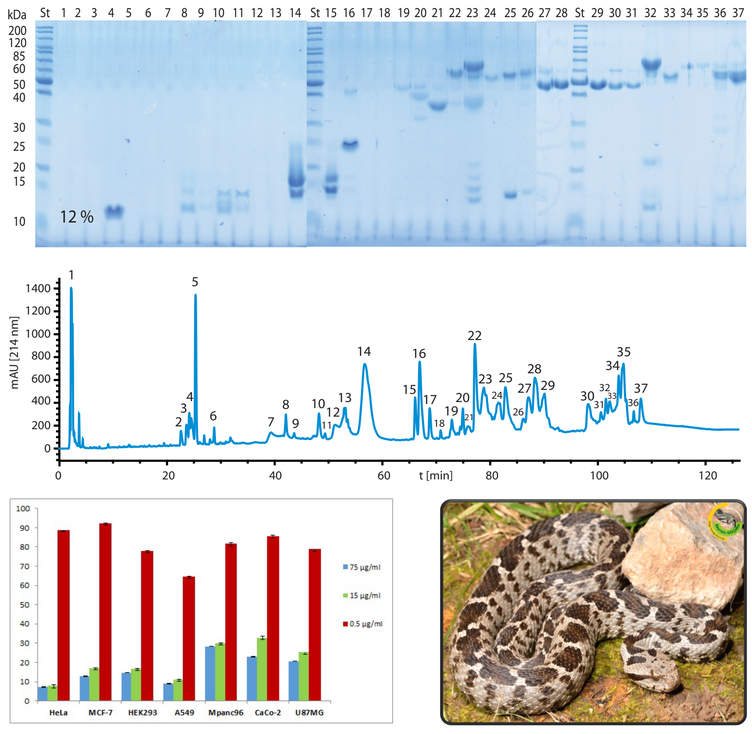
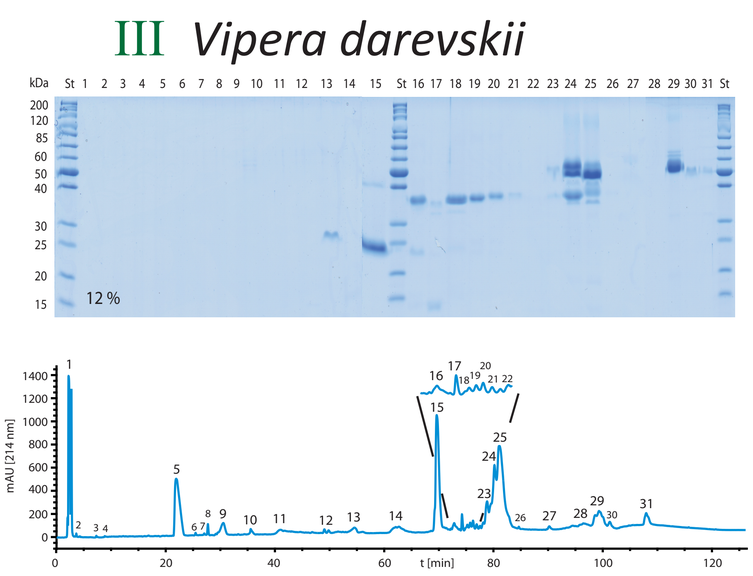


Figure 5. Representative RP-HPLC profile and coomassie stained SDS-gels of ***Montivipera bulgardahica***, Cytotoxic activity of crude venom against various cancer cellines and *Montivipera bulgardahica* in its natural habitat (picture taken ba Bayram Göçmen)

III *Vipera darevskii*



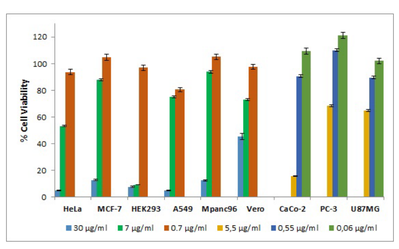


Figure 6. Representative RP-HPLC profile and coomassie stained SDS-gels of ***Vipera darevskii***, Cytotoxic activity of crude venom against various cancer cellines and *Vipera darevsii* in its natural habitat (picture taken ba Bayram Göçmen)

REFERENCES

1 Göçmen, B., Heiss, P., Petras, D., Nalbantsoy, A., Süssmuth, R.D., Mass spectrometry guided venom profiling and bioactivity screening of the Anatolian Meadow Viper, Vipera anatolica, Toxicon (2015), doi: 10.1016/j.toxicon.2015.09.013.

CONTACT INFORMATION

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