TopSpec - 829157

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Young scientist TopSpec technology workshop

Content:

Authors: Susanna Lundström, Roman Zubarev, Karolinska Institutet, Jan Commandeur, MS Vision

Strategy

Aim: The aim with the Young scientist TopSpec technology workshop was to make the scientific community aware of the new technology. In order to make the event more attractive we invited additional prominent external speakers to the event and made it the main highlight and focus on Day 2 (January 17, 2022) at the Janeiro-na-Madeira Winter Summer School 2022 hybrid event.

Advertisement: The event was advertised on the TopSpec webpage, LinkedIN, and Twitter as well as by the partners own media sources and contacts, and at the Janeiro-na-Madeira Winter Summer School 2022 webpage. (https://janeiro-na-madeira.mozello.com/).

Workshop

Speakers and Agenda: For the “TopSpec” day of the event, lectures by TopSpec partners, Dimitris Papanastasiou (Fasmatech), Yury Tsybin (Spectroswiss), Alexander Makarov (Thermo Fisher) and Julia Chamot-Rooke (Pasteur Institute) were held. Additionally, Neil Kelleher (North Western University) and Ying Ge (University of Wisconsin) were invited to speak. For more details see agenda (**Figure 1**). Furthermore, the opening lecture of the event was held by TopSpec partner Roman Zubarev (Karolinska Institutet). In **Figure 2** and **Figure 3** pictures and screenshots taken during the presentations are shown.

Monday 17th January-Top-down MS / TopSpec

GMT (CET -1h)	
09:00- 11.00	TopSpec consortium meeting
11.00	Coffee break
	Zoom Meeting Link
11.20	Latest Omnitrap design and performance, Dimitris Papanastasiou, Fasmatech, Athens, Greece
12:00	Top-down MS signal detection and processing, Yury Tsybin, SpectroSwiss, Lausanne, Switzerland
13:00	Lunch
	Zoom Meeting Link
14:00	Novel developments in Orbitrap Mass Spectrometry, Alexander Makarov, Thermo Fisher Scientific, Bremen, Germany
14:40	Top-down mass spectrometry at Institut Pasteur, Julia Chamot-Rooke, Institut Pasteur, Paris, France
15:20	Coffee break
15:30	High-resolution Serology by Ig-MS: Readout of the SARS-CoV-2 Antibody Repertoire using Individual Ion Mass Spectrometry, Neil Kelleher, Northwestern University, Chicago, IL, USA
16:20	New Strategies in Top-down Proteomics for Precision Medicine, Ying Ge, University of Wisconsin, Madison, WI, US
17:00	General discussion

Figure 1. Agenda of the day dedicated to TopSpec and TopDown MS at the Janeiro-na-Madeira Winter Summer School. The image is taken from the official homepage.

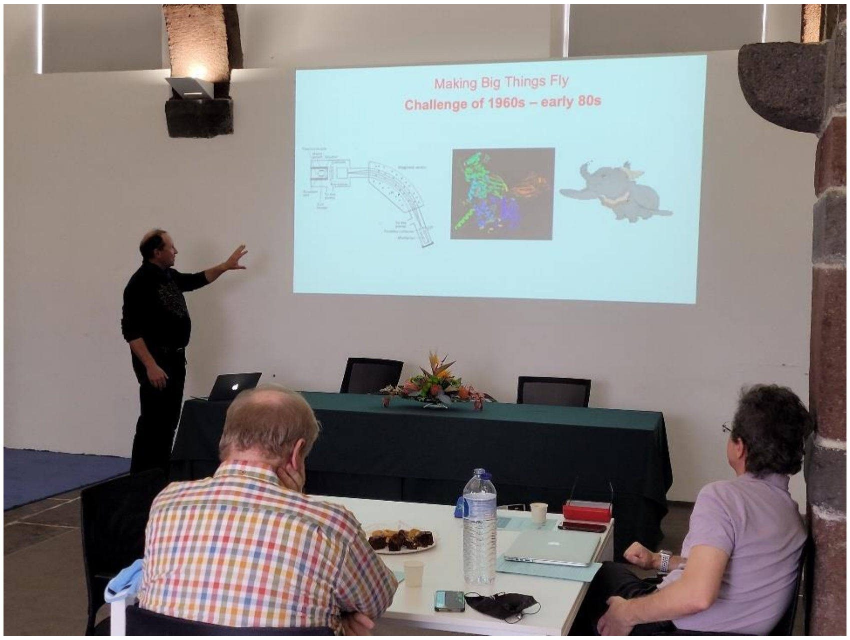
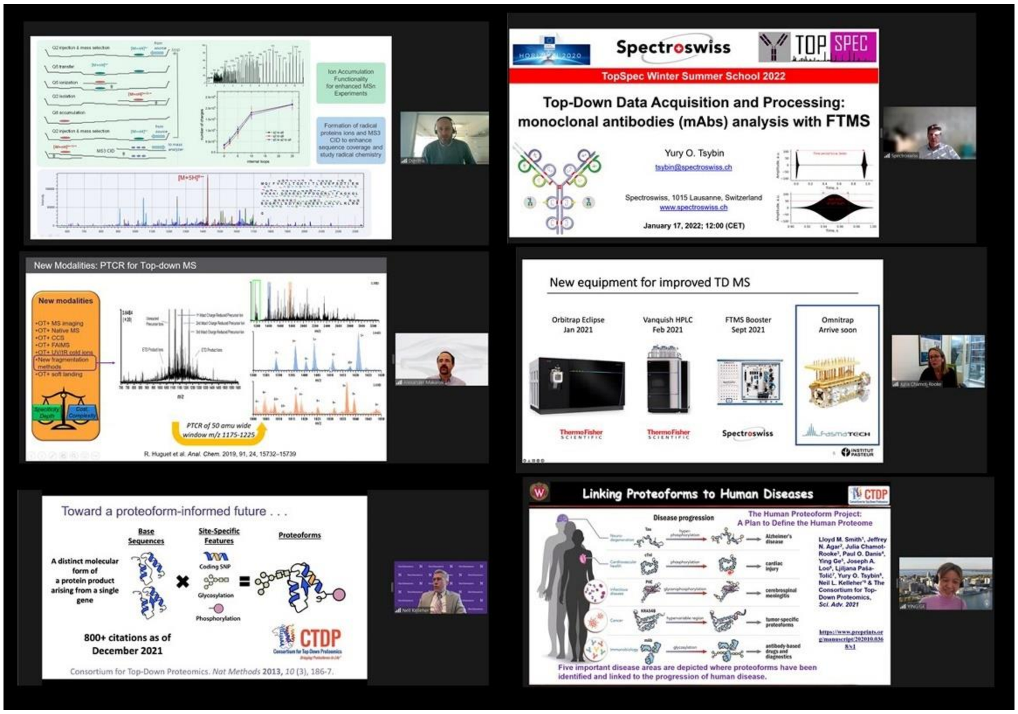


Figure 2. Opening lecture by Roman Zubarev, (Karolinska Institutet).



Ion Accumulation Functionality for enhanced MSn Experiments

Formation of radical proteins ions and MS3 CID to enhance sequence coverage and study radical chemistry

Spectroswiss | TOP SPEC

TopSpec Winter Summer School 2022

Top-Down Data Acquisition and Processing: monoclonal antibodies (mAbs) analysis with FTMS

Yury O. Tsybin
tsybin@spectroswiss.ch

Spectroswiss, 1015 Lausanne, Switzerland
www.spectroswiss.ch

January 17, 2022; 12:00 (CET)

New Modalities: PTCR for Top-down MS

New modalities
- OT+ MS imaging
- OT+ Native MS
- OT+ CCS
- OT+ FAIMS
- OT+ UV/IR cold ions
- New fragmentation methods
- OT+ soft landing

PTCR of 50 amu wide window m/z 1175-1225

R. Huguet et al. *Anal. Chem.* 2019, 91, 24, 15732–15739

New equipment for improved TD MS

Orbitrap Eclipse Jan 2021	Vanquish HPLC Feb 2021	FTMS Booster Sept 2021	Omnitrap Arrive soon
ThermoFisher Scientific	ThermoFisher Scientific	Spectroswiss	FasmaTech

Toward a proteoform-informed future . . .

Base Sequences × Site-Specific Features = Proteoforms

A distinct molecular form of a protein product arising from a single gene

Coding SNP
Glycosylation
Phosphorylation

800+ citations as of December 2021

CTDP Consortium for Top-Down Proteomics

Consortium for Top-Down Proteomics. *Nat Methods* **2013**, *10* (3), 186-7.

Linking Proteoforms to Human Diseases

Disease progression

The Human Proteoform Project: A Plan to Define the Human Proteome

Lloyd M. Smith[1], Jeffrey N. Agar[2], Julia Chamot-Rooke[3], Paul O. Danis[4], Ying Ge[5], Joseph A. Loo[6], Ljiljana Paša-Tolić[7], Yury O. Tsybin[8], Neil L. Kelleher[9] & The Consortium for Top-Down Proteomics, *Sci. Adv.* 2021

https://www.preprints.org/manuscript/202010.0368/v1

Five important disease areas are depicted where proteoforms have been identified and linked to the progression of human disease.

Figure 3. Screen shots from the TopSpec- and top-down MS lectures at the event. Speakers: Dimitris Papanastasiou (Fasmatech), Yury Tsybin (Spectroswiss), Alexander Makarov (Thermo Fisher), Julia Chamot-Rooke (Pasteur Institute), Neil Kelleher (North Western University) and Ying Ge (University of Wisconsin).

Presentations:

<u>Roman Zubarev (Karolinska Institutet):</u> *Janeiro-na-Madeira Event 2022 - Welcome and Introduction*

Part I: Introduction of the meeting. *Janeiro-na-Madeira* is a hybrid event combining consortia meetings (TopSpec, ARIADNE), community building event (Deamidation), and Winter school (Top-Down mass spectrometry, Single cell proteomics, Chemical proteomics), as well as diverse lectures on suitable topics (Madeira wine research). The goal is to establish Funchal as a site of scientific meetings in a pleasant environment, providing ripe opportunity for communication. Part II: The second part of the talk was devoted to contribution to science of two great mass spectrometrists who passed away in 2021: Ron Macfarlane and Fred McLafferty. It is shown that they pursued novel ideas that met resistance in the beginning but became popular with time.

<u>Dimitris Papanastasiou (Fasmatech):</u> *Latest Omnitrap design and performance*.

The talk was dedicated to the omnitrap technology developed on orbitrap-based mass spectrometry. The basic features of the technology were discussed, and the multidimensional multiple-stage tandem MS capabilities were described. The content of the article introducing the Omnitrap technology to the mass spec community was presented, where complete sequence coverage for unfolded ubiquitin is demonstrated with five different ion-activation dissociation methods. The EID capabilities available on chromatographic time scales were also presented as well as the strong electron meta-ionization of proteins accomplished at the higher electron energies. Finally, the first MS4 experiment performed with intact mAbs achieved during the course of the TopSpec project was discussed in greater detail. Record levels of sequence coverage obtained for the light chain of trastuzumab sprayed at denaturing conditions were reported. Finally, latest developments in data processing software and a new de novo algorithm dedicated to top down proteomics were shown, together with a new RF ion mobility drift cell connected in series with the Omnitrap platform.

<u>Yury Tsybin (Spectroswiss):</u> *Top-down MS signal detection and processing*.

The lecture covered both didactic aspect and recent research results in the workflows for data processing and data analysis employed for the structural analysis of monoclonal antibodies (mAbs) with FTMS. The presentation started with a history of the subject, outlining the inception of the field of mAb analysis with mass spectrometry and tandem mass spectrometry. Then an overview of the mAb analysis with MS/MS in the modern days was given, providing a summary of the 2022 top-down MS toolbox. After this introduction, we formulated and proceeded to discuss in more detail the following Top-Down FTMS data acquisition concepts:

- Sample preparation: desalting, deglycosylation, S-S bond reduction, subunits
- Sample introduction: direct infusion (ESI and MALDI), RPLC, SEC, CE
- MS and MS/MS data acquisition modes: denaturing and native
- MS/MS methods: ECD/ETD, CID, UVPD, MALDI ISD, etc.
- Experimental settings: resolution, number of ions, microscans and technical replicates
- Data types selection: reduced and unreduced data

In line with the outline above, we then formulated and proceeded to discuss in more detail the Top-Down FTMS data processing concepts:

- Data processing and data analysis software: accuracy, speed, and automation

- Mass spectra generation from time-domain transients: isotopic beats
- Resolution needs: intact mass and product ion analysis, including ultra-low resolution approach
- Mass accuracy needs: terminal and internal product ions
- Sensitivity: horizontal and vertical data averaging
- Intact mass analysis: to deconvolve or not?
- Product ion distribution analysis: to deconvolve or not?
- Product ion distribution complexity reduction: proton transfer reaction, ion mobility
- Charge state information extraction from time-domain transients: CDMS
- De novo analysis of top-down MS data

We concluded with the following 10 tips for top-down FTMS data analysis:

- Simulate your MS and MS/MS data. The FTMS Simulator is an option
- Use the unreduced data (full profile aFT > full profile eFT > reduced profile eFT)
- Resolution: from ultra-low and ultra-high. Simulations may help to estimate the needs
- Mass accuracy: to improve the current levels! Do internal re-calibration if possible
- Sensitivity: use technical replicates for vertical data averaging, and/or multiple fills
- Deconvolution: try also software tools that avoid it by doing a direct peak matching for both MS and MS/MS data – TDValidator, AutoVectis, Peak-by-Peak BioPharma
- Combined data from diverse MS/MS methods: Eclipse, QE/Exploris + Omnitrap
- Simplify product ion distributions – use proton transfer reaction (or PTCR)
- Validate and enhance your top-down data with middle-down, bottom-up, and de novo
- Try single (individual) ion counting (I2MS, CDMS) for your complex samples

Alexander Makarov (Thermo Fisher): *Novel developments in Orbitrap Mass Spectrometry*.

This talk started from a didactic summary of high-resolution accurate mass (HR/AM) analysis using Orbitrap mass spectrometry. Basic principles of this technology were directly linked to analytical performance metrics, with special attention paid to analysis of proteins – the objective of TopSpec project.

Improvement of protein analysis with Orbitrap mass spectrometry remains an area of intense research. Specifically, latest frontiers and analytical utility were presented for such diverse avenues as ultra-high resolving power, ultra-high mass analysis, multi-stage native -omics, new technique of proton-transfer charge reduction, high-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS), different approaches to charge detection mass spectrometry, boxcar high-dynamic range scanning, phase-constrained signal deconvolution method for improving resolution. Many of these directions of improvement pose unique challenges to mass spectrometry and drive the deep re-thinking of principles earlier validated on small molecules and peptides.

Julia Chamot-Rooke (Pasteur Institute): *Top-down mass spectrometry at Institut Pasteur*.

Julia Chamot-Rooke started with an update on the instrumentation newly installed in her lab for the development of top-down proteomics and the different ongoing projects and associated scientists. She further described the added-value of top-down proteomics for the analysis of challenging and highly modified proteins that are called tubulins and that are involved in many important biological processes such as neurodegeneration and infertility. The talk continued with a description of the TDFragmapper software, developed by her group, that allows an easy visualization of top-down MS/MS data. It ended up with perspectives in the field including the imminent installation of the omnitrap from Fasmatech for the analysis of intact antibodies.

Neil Kelleher (Northwestern University): *High-resolution Serology by Ig-MS: Readout of the SARS-CoV-2 Antibody Repertoire using Individual Ion Mass Spectrometry.*

A novel serological readout approach (Ig-MS) was presented. Ig-MS captures the immunoglobulin (Ig) repertoire at molecular resolution, including entire variable regions in Ig light and heavy chains. Ig-MS uses recent advances in protein mass spectrometry (MS) for multiparametric readout of antibodies, with new metrics like Ion Titer (IT) and Degree of Clonality (DoC) capturing the heterogeneity and relative abundance of individual clones without sequencing of B cells.

Ying Ge (University of Wisconsin): *New Strategies in Top-down Proteomics for Precision Medicine.*

In the past decade, top-down proteomics has experienced rapid growth benefiting from groundbreaking technological advances, which have begun to reveal the potential of top-down proteomics for understanding basic biological functions, unraveling disease mechanisms, and discovering new biomarkers. New strategies in Top-down Proteomics for precision medicine were presented and discussed.

Participants

In total 78 individuals participated at the event. A majority (86%) participated online and were from the academia (72%). The event was visited by individuals from 39 different affiliations listed in **Table 1** and 14 different countries listed in **Table 2**. A complete list of the participants is given in **Table 3**. Even though the pandemic made it difficult to travel to the destination, which reduced the opportunity for a majority to obtain the valuable direct discussions and idea exchanges that only direct face to face interactions can bring, we believe that the event was a success. In **Figure 4** and in **Figure 5** pictures from the event on site are given.

Figure 4. Alexander Makarov (PI, Thermo Fisher) and Zhaowei Meng (PhD student, Karolinska Institutet) discussing the TopSpec project.

Figure 5. On site participants. Akos Vegari, Muriel Priault, Susanna Lundström, Zhaowei Meng, Roman Zubarev, Karl Mechtler, Massimiliano Gaetani, José Carlos Marques and Alexander Makarov.

Table 1. Affiliations of the participants.

Affiliation	**Paricipants**
Biomotif	2
European Molecular Biology Laboratory (EMBL)	1
Fasmatech	6
Fossil Ion Tech	1
Instituto Superior Técnico (IST)	1
Technical University of Munich	1
Thermo Fisher	5
University of Bordeaux	1
University of Wisconsin	1
Brock University	1
Centre national de la recherche scientifique (CNRS)	1
Collège de France	1
CSSB-HPI	1
Delft University of Technology	1
Federal University of Mato Grosso do Sul	1
German Cancer Research Center	1
Harvard University	1
Institute of Molecular Pathology	1
Institute Pasteur	2
Karolinska Institutet	14
Leibniz Institute for Experimental Virology (HPI)	1
Max Planck Institute	1
Nordic Preclinical Sciences	1
Northeastern University	2
Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA)	1
Spectrowiss	2
TNTU	1
Universidad de Buenos Aires	1
University of Aveiro	1
University of Barcelona	1
University of Copenhagen	2
University of Geneva	1
University of Hamburg	1
University of Lisbon	5
University of Madeira	1
Uppsala University	1
Utrecht University	1
Vienna BioCenter (VBC)	1
European Molecular Biology Laboratory (EMBL)	1
Unknown	9

Table 2. Nationalities of the participants.

Country	**Participants**
Sweden	18
Germany	13
Portugal	10
Greece	7
France	4
USA	4
Spain	3
Switzerland	3
Denmark	2
Austria	2
Australia	2
UK	1
Argentina	1
Canada	1
Unknown	7

Table 3. Participant list.

Participants	**Affiliation**	**Country**
Akos Vegvari	Karolinska Institutet	Sweden
Albin Lundin	Uppsala University	Sweden
Alexander Makarov	Thermo Fisher	Germany
Alexandros Lekkas	Fasmatech	Greece
Amir Ata Sei	Karolinska Institutet	Sweden
Ana Marques	Instituto Superior Técnico (IST)	Portugal
Andrey Grinfeld	Thermo Fisher	Germany
Ann H. Lyu	Biomotif	Sweden
Bogdan Budnik	Harvard University	USA
Carlos Cordeiro	University of Lisbon	Portugal
Charlotte Uetrecht	University of Hamburg	Germany
Christian Beuch	Karolinska Institutet	Sweden
David Kilgour	TNTU	UK
Dimitris Papanastasiou	Fasmatech	Greece
Eric Wapelhorst	Thermo Fisher	Germany
Esben Trabjerg	University of Copenhagen	Denmark
Eugene Moskovets	Northeastern University	USA
Francisco Traquete	University of Lisbon	Portugal
George Biskos	Delft University of Technology	Greece
Gerhard Durnberger	Vienna BioCenter (VBC)	Austria
Giorgos Alevizos	Fasmatech	Greece
Guillermo Vidal-de-Miguel	Fossil Ion Tech	Spain
Hassan Gharibi	Karolinska Institutet	Sweden
Herbert Waldmann	Max Planck Institute	Germany
Jean-Pierre Changeux	Collège de France	France
Jeroen Krijgsveld	German Cancer Research Center	Germany
Jijing Janet	Karolinska Institutet	Sweden
Joao Pinto	Federal University of Mato Grosso do Sul	Portugal
Joao Luz	University of Lisbon	Portugal
José Carlos Marques	University of Madeira	Portugal
Julia Chamot-Rooke	Institute Pasteur	France
Karl Mechtler	Institute of Molecular Pathology	Austria
Kasper Rand	University from Kopenhagen	Denmark
Kostya Aizikov	Thermo Fisher	Germany

Kuster Bernhard	Technical University of Munich	Germany
Kyle Fort	Thermo Fisher	Germany
Lars Thiede	CSSB-HPI	Germany
Leonardo Alonso	Universidad de Buenos Aires	Argentina
Linnea Yuan Andersson	Karolinska Institutet	Sweden
Mariana Louro	University of Lisbon	Portugal
Mariangela Kosmopoulou	Fasmatech	Greece
Marie Ståhlberg	Karolinska Institutet	Sweden
Massimiliano Gaetani	Karolinska Institutet	Sweden
Megan Cornelius		Australia
Mikhail Savitski	European Molecular Biology Laboratory (EMBL)	Germany
Marcos Minarro	Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA)	Spain
Monica Soeiro	University of Lisbon	Portugal
Muriel Priault	University of Bordeaux	Portugal
Konstantin Nagornov	Spectroswiss	Switzerland
Neil Kelleher	Northwestern University	USA
Newman Siu Kwan SZE	Brock University	Canada
Nikolos Ioannis	Fasmatech	Greece
Olga Lytovchenko	Karolinska Institutet	Sweden
Pedro Fernandes	University of Aveiro	Portugal
Rodolphe Antoine	Centre national de la recherche scientifique (CNRS)	France
Roman Zubarev	Karolinska Institutet	Sweden
Ronja Pogan	Leibniz Institute for Experimental Virology (HPI)	Germany
Sergey Girel	University of Geneva	Switzerland
Sourav. Pr Mukherjee	Nordic Preclinical Sciences	Sweden
Susanna Lundström	Karolinska Institutet	Sweden
Thanos Smyrnakis	Fasmatech	Greece
Thorleif Lavold	Biomotif	Sweden
Tingting FU	Institute Pasteur	France
Tobias Wörner	Utrecht University	Germany
Varbina Ivanova	University of Barcelona	Spain
Xuepei Zhang	Karolinska Institutet	Sweden
Ying Ge	University of Wisconsin	USA
Yury Tsybin	Spectrowiss	Switzerland
Zhaowei Meng	Karolinska Institutet	Sweden
Ziqing Chen	Karolinska Institutet	Sweden

Zygmund Sieradzki		Australia
Booster ?	-	-
Ilias ?	-	-
Janet ?	-	-
Mike ?	-	-
Serena ?	-	-
Sw?	-	-
vivanova ?	-	-