EUROPEAN COMMISSION
Research Executive Agency
Director

AMENDMENT Reference No AMD-829157-12

Grant Agreement number: 829157 — Next generation precision antibody profiling - from science fiction to reality (TopSpec)

The parties agree to amend the Grant Agreement as follows ('**Amendment**'):

1. Change of Annex 1 (description of the action)

Annex 1 is changed and replaced by the Annex 1 attached to this Amendment.

2. Change of the action's duration

The duration of the action in **Article 3** is changed to 42 months.

3. Change of the reporting periods

The reporting periods are changed.

This implies the **following changes** to the Grant Agreement:

- The reporting periods in **Article 20.2** are replaced by:

  - RP1: from month 1 to month 12
  - RP2: from month 13 to month 42

All other provisions of the Grant Agreement and its Annexes remain unchanged.

This Amendment **enters into force** on the day of the last signature.

This Amendment **takes effect** on the date on which the amendment enters into force, except where a different date has been agreed by the parties (for one or more changes).

Please inform the other members of the consortium of the Amendment.

SIGNATURES

For the coordinator

For the Agency

Enclosures:

Annex 1

EUROPEAN COMMISSION
Research Executive Agency

The Director

ANNEX 1 (part A)

Research and Innovation action

NUMBER — 829157 — TopSpec

Table of Contents

1.1. The project summary

Project Number [1]	829157	Project Acronym [2]	TopSpec

One form per project

General information	
Project title [3]	Next generation precision antibody profiling - from science fiction to reality
Starting date [4]	01/01/2019
Duration in months [5]	42
Call (part) identifier [6]	H2020-FETOPEN-2018-2019-2020-01
Topic	FETOPEN-01-2018-2019-2020 FET-Open Challenging Current Thinking
Fixed EC Keywords	Spectroscopic and spectrometric techniques, Proteomics, Molecular biology and interactions, Proteomics in biochemistry, Mass Spectrometry, Instrumentation and Instrumental techniques
Free keywords	Antibody profiling, immunotherapeutics, Mass spectrometry, Tandem mass spectrometry

Abstract [7]

One of the major challenges of modern medicine is to understand how the human organism defends itself against invasions and diseases. The biggest mystery is the human immune system, and understanding this ultimately requires knowledge of the sequence repertoire of human antibodies and their respective antigens. The purpose of the TopSpec project is to be the first in the world to solve this challenge, opening up opportunities in medical research and drug development that are today only dreamt about. We will create a breakthrough technology that will revolutionize academic, clinical and industrial proteomics and dramatically advance the development of new generation antibody- and protein-based therapeutics. Antibodies represent the most sophisticated line of natural defense against disease. Knowing exactly which antibodies are produced in response to a particular disease enables us not only to better understand the cause of the disease but also to provide new-generation cures in the form of personalized therapeutic antibodies. The limiting factor for this to truly be achieved is to find a way to analyze and sequence large molecules in the gas phase, and this represents a formidable challenge. The TopSpec project will develop ground-breaking TOP-down tandem mass SPECtrometry (MS/MS) approaches based on novel radical gas-phase ion-electron and ion-atom reactions, and implement them on a unique, hyphenated, ultrahigh-resolution MS platform. Another “killer innovation” is the ability to greatly simplify MS/MS spectra of large molecules by adding another dimension of separation – collisional cross-sections of fragment ions using two parallel approaches. TopSpec will be the first project to implement de-convolution of massively overlapping isotopic clusters, solving one of the greatest challenges in top-down MS of large molecules.

1.2. List of Beneficiaries

Project Number [1]	829157	Project Acronym [2]	TopSpec

List of Beneficiaries

No	Name	Short name	Country	Project entry date[8]	Project exit date
1	KAROLINSKA INSTITUTET	KI	Sweden		
2	FASMATECH EPISTIMONIKI KAI TECHNOLOGIKI ANONYMI ETAIREIA	FASMATECH SA	Greece		
3	THERMO FISHER SCIENTIFIC (BREMEN) GMBH	THERMO FISHER	Germany		
4	SPECTROSWISS SARL	SPECTROSWISS	Switzerland		
5	BIOMOTIF AB	Biomotif AB	Sweden		
6	THE NOTTINGHAM TRENT UNIVERSITY	TNTU	United Kingdom		
7	INSTITUT PASTEUR	IP	France		
8	SPECTROMETRY VISION BV	MS VISION	Netherlands		

1.3. Workplan Tables - Detailed implementation

1.3.1. WT1 List of work packages

WP Number[9]	WP Title	Lead beneficiary[10]	Person-months[11]	Start month[12]	End month[13]
WP1	Omnitrap development and testing	2 - FASMATECH SA	42.00	1	42
WP2	Implementation of CAD, ECD, HECD, UV, IR PD, and EID MS/MS techniques in Omnitrap	1 - KI	42.00	1	30
WP3	Development and application of H-atom bombardment (HAB) MS/MS techniques	2 - FASMATECH SA	85.00	1	30
WP4	Development and application of Coulomb explosion MS/MS technique	1 - KI	49.00	18	39
WP5	Development of pI-Trap-ESI combination	5 - Biomotif AB	42.00	15	42
WP6	Modification of the Orbitrap mass spectrometer	3 - THERMO FISHER	38.00	2	42
WP7	Signal detection and data processing	4 - SPECTROSWISS	108.00	1	40
WP8	Dissemination, Communication & Exploitation	8 - MS VISION	71.00	3	42
WP9	Project Management and Administration	1 - KI	72.00	1	42
		Total	549.00		

1.3.2. WT2 list of deliverables

Deliverable Number[14]	Deliverable Title	WP number[9]	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D1.1	Two fully equipped Omnitraps & one IMS installed	WP1	2 - FASMATECH SA	Other	Public	28
D1.2	Modified Omnitraps with updated software	WP1	1 - KI	Other	Public	39
D1.3	Fully serviced, functioning Omnitraps & IMS	WP1	2 - FASMATECH SA	Other	Public	42
D2.1	in situ testing of the optimized CAD MS/ MS protocol	WP2	1 - KI	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	18
D2.2	Protocol of in situ testing of the optimized CAD	WP2	1 - KI	Report	Confidential, only for members of the consortium (including the Commission Services)	20
D2.3	in situ testing optimized ECD, HECD and EID MS/MS	WP2	1 - KI	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	30
D3.1	Prototype of the HAB gun installed and tested protocol	WP3	2 - FASMATECH SA	Other	Confidential, only for members of the consortium (including the Commission Services)	15
D3.2	Optimized HAB guns installed and tested - protocols	WP3	2 - FASMATECH SA	Other	Confidential, only for members of the consortium (including the Commission Services)	30
D4.1	Protocol- CED gun prototype installed and tested	WP4	1 - KI	Other	Confidential, only for members of the consortium (including the Commission Services)	30
D4.2	Protocols: CED guns installed and tested	WP4	1 - KI	Other	Confidential, only for members of the consortium	39

Deliverable Number[14]	Deliverable Title	WP number[9]	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
					(including the Commission Services)	
D5.1	Prototype pI-Trap-ESI installed and tested– protocol	WP5	5 - Biomotif AB	Other	Public	20
D5.2	Two tested, optimized pI-Trap-ESI installed and tested	WP5	5 - Biomotif AB	Other	Public	42
D6.1	Installation of Q Exactive instrument for Omnitrap development	WP6	3 - THERMO FISHER	Other	Confidential, only for members of the consortium (including the Commission Services)	12
D6.2	Modified Orbitrap Q Exactive HF X installed	WP6	3 - THERMO FISHER	Other	Public	42
D7.1	Two Prototype FTMS Booster installed and tested-protocol	WP7	4 - SPECTROSWISS	Other	Confidential, only for members of the consortium (including the Commission Services)	30
D7.2	Top-down analysis software	WP7	4 - SPECTROSWISS	Other	Confidential, only for members of the consortium (including the Commission Services)	35
D7.3	Optimized FTMS Boosters test protocols	WP7	4 - SPECTROSWISS	Other	Confidential, only for members of the consortium (including the Commission Services)	40
D8.1	IP protection strategy finalized	WP8	8 - MS VISION	Other	Public	3
D8.2	Draft Exploitation plan and Business strategy document	WP8	8 - MS VISION	Report	Public	12
D8.3	Young scientist TopSpec technology workshop	WP8	8 - MS VISION	Other	Public	39
D8.4	Public demonstrations of TopSpec technology	WP8	8 - MS VISION	Report	Public	39
D8.5	Scientific reports and publications	WP8	8 - MS VISION	Report	Public	42

Deliverable Number[14]	Deliverable Title	WP number[9]	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D8.6	Exploitation plan and Business strategy document	WP8	8 - MS VISION	Report	Confidential, only for members of the consortium (including the Commission Services)	42
D9.1	Logo and Website launch and public accessibility	WP9	1 - KI	Other	Public	2
D9.2	Data management plan	WP9	1 - KI	ORDP: Open Research Data Pilot	Public	6
D9.3	Technical/scientific review meeting documents	WP9	1 - KI	Report	Confidential, only for members of the consortium (including the Commission Services)	13
D9.4	Scientific review meeting documents	WP9	1 - KI	Report	Confidential, only for members of the consortium (including the Commission Services)	30
D9.5	Final: Technical/ scientific review meeting documents	WP9	1 - KI	Report	Confidential, only for members of the consortium (including the Commission Services)	42

1.3.3. WT3 Work package descriptions

Work package number [9]	WP1	Lead beneficiary [10]	2 - FASMATECH SA
Work package title	Omnitrap development and testing		
Start month	1	End month	42

Objectives

To develop an Omnitrap with advanced ion transfer and manipulation capabilities.

Description of work and role of partners

WP1 - Omnitrap development and testing [Months: 1-42]
FASMATECH SA, THERMO FISHER
Task 1.1 Mechanical design & testing (FASM, M1-M4): A novel design will be produced with reduced capacitive coupling, eliminated resistor over-heating in vacuum, precision machining and new pulsed beam source. The reduced capacitive coupling will be achieved by new hyperbolic electrode structures and will ensure that higher order-field components and associated non-linear resonances will not be present and affect performance. These studies will be performed using ion optical simulation tools. Overheating in vacuum will be accomplished by a introducing a multi-pin spring- contact feedthrough distributing RF and DC signals on a two-level in-vacuum pcb for driving the omnitrap. The new pulsed electron beam source will utilize a new set of high voltage electron optics to counterbalance the string effects of space charge and also a high voltage pulser unit synchronized with the main RF drive and controlled through the FPGA unit to create pulses of desired length.
Task 1.2 Ion optical simulations (FASM, M1-M5): Effects of waveform non-idealities on ion isolation will be investigated, as well as ion isolation using resolving DC and variations of the RF duty cycle as an alternative. These studies will rely on precise measurements of ion stability conditions using RF-DC scanning methods to identify sharp boundaries which can be utilized for ejecting lower or higher m/z values relative to the window of interest. A calibration table will be generated to automate the process of m/z selection and also allow the user to define the width of the window.
Task 1.3 Electronics design & testing (FASM, M1-M7): An improved rectangular RF drive generator with voltage pulse stability of < 3% and minimized jitter will be developed. The target value is 250V0p at 2MHz, but efforts to push electronics beyond this threshold will be made to enhance isolation, trapping efficiency and increase the number of ions that can be stored in the trap by reducing the undesired effects of space charge. Optimization of sweep algorithms will be performed to allow for multi-notch isolation experiments. Finally, a new FPGA platform will be implemented that will allow precise control of trigger signals which are essential for synchronizing all the complex functionality available in the omnitrap.
Task 1.4 Mechanical & vacuum assembly (FASM, M7-M18): Assembly of two Omnitraps and one IMS to meet tolerance specifications together with vacuum testing and related gas load during operation of the pulse valve systems will be carried out. Inspection tests of tolerances achieved will be performed and a residual gas analyzer will also be employed to monitor the quality of the gas. Modifications to the original omnitrap design will be considered to facilitate easy removal and cleaning of the electron optics assembly.
Task 1.5 Design control software (FASM, M6-18): Production of instruction sequence lists for basic experiments, bundle modes development and implementation of new automatic modes of operation to control vacuum state. Each bundle will also be accessible to the user to perform in-depth optimization of the extended functionality of the trap. Automated m/z isolation procedures will be achieved by precise tuning of RF amplitude, frequency and duty cycle of the waveforms. A series of dipolar excitation experiments will also allow for precise measurements of ion secular frequency. All this functionality will be available through the sequence list of the omnitrap software.
Task 1.6 Electronics testing & synchronization (FASM, M10-M18): Communication board and software design to synchronize ion transfer from and to the C-trap of the Orbitrap. These experiments involve optimization of cooling pressures and ion axial energy, together with gas pulsing performed in the omnitrap to receive and thermalize high mass protein ions. The experiments will also involve frequency and RF amplitude optimization in additional to the axial DC profile and related DC switching of electrodes used to gate pulses of ions.
Task 1.7 Installation & instructions in situ (FASM, M14,23,28): The Omnitrap + IMS will be installed in Stockholm at KI and the standalone Omnitrap in Paris at IP. Training of local research personnel and students will be executed. A detailed manual of operation will also be provided. Training in Athens before instrument shipping is also an option and will be discussed between partners.

Task 1.8 Modifications and optimization in situ (FASM, TF, M18-19, 26,30,33): Necessary modifications will be performed as per request of the researchers at KI and IP to improve and optimize Omnitrap performance. These optimizations will be related to the operation of the hyperthermal H gun and the bright electron source for improving sequence information.
Task 1.9 Maintenance and servicing in situ (FASM, TF, M18,21,25,29,33): will be performed in both locations to ensure fault-free performance. Clean-up, consumable replacement, other preventive maintenance during the course of this project.

Participation per Partner

Partner number and short name	WP1 effort
2 - FASMATECH SA	39.00
3 - THERMO FISHER	3.00
Total	42.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D1.1	Two fully equipped Omnitraps & one IMS installed	2 - FASMATECH SA	Other	Public	28
D1.2	Modified Omnitraps with updated software	1 - KI	Other	Public	39
D1.3	Fully serviced, functioning Omnitraps & IMS	2 - FASMATECH SA	Other	Public	42

Description of deliverables

D1.1. Two Omnitraps + one IMS fully equipped with driving electronics, gas lines and pulse valves and control software designed, constructed and installed
D1.2. Modified and optimized Omnitraps with updated software
D1.3. Fully serviced, functioning Omnitraps

D1.1 : Two fully equipped Omnitraps & one IMS installed [28]

Two Omnitraps + one IMS fully equipped with driving electronics, gas lines and pulse

D1.2 : Modified Omnitraps with updated software [39]

Modified and optimized Omnitraps with updated software (month 33).

D1.3 : Fully serviced, functioning Omnitraps & IMS [42]

Fully serviced, functioning Omnitraps.

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS2	Omnitraps & IMS Electronics design	2 - FASMATECH SA	12	Omnitraps & IMS Electronics design finalized
MS4	Omnitraps & IMS P.O.s sent to suppliers	2 - FASMATECH SA	12	Omnitraps & IMS P.O.s sent to suppliers
MS5	Omnitraps & IMS Mechanical design	2 - FASMATECH SA	12	Omnitraps & IMS Mechanical design finalised
MS9	Interfacing pI-Trap-Orbitrap OMNI-ORBI combination	2 - FASMATECH SA	32	Capacity to sequence intact proteins

Work package number [9]	WP2	Lead beneficiary [10]	1 - KI
Work package title	Implementation of CAD, ECD, HECD, UV, IR PD, and EID MS/MS techniques in Omnitrap		
Start month	1	End month	30

Objectives

To implement and test in Omnitrap ion isolation and collision-based fragmentation techniques (CAD) and electron-based fragmentation techniques ECD, HECD and EID, as well as IR and UV photo-dissociation.

Description of work and role of partners

WP2 - Implementation of CAD, ECD, HECD, UV, IR PD, and EID MS/MS techniques in Omnitrap [Months: 1-30]
KI, FASMATECH SA, THERMO FISHER , TNTU, IP
Task 2.1 Testing in situ ion isolation techniques (KI, FASM, IP, NTU, M15-17).
- Determine the width of the m/z window at <50% ion loss using different methods of ion isolation in the Omnitrap.
- Optimize the parameters of ion isolation, including the frequency and amplitude of the excitation voltage as a function of ion's m/z, to obtain the highest possible efficiency of ion isolation.
- Repeat the optimization for 2-, 3-, and multi-notch isolation.

Task 2.2 Testing in situ collision-activated, UV and IR dissociation reactions (KI, FASM, IP, NTU, M16-18)
- testing using ubiquitin and cytochrome C molecular ions the efficiency and sequence coverage obtained using collision-activated dissociation (CAD) in the Omnitrap; determine maximum efficiency as a ratio of the total fragment charge over precursor charge.
- testing using ubiquitin and cytochrome C molecular ions the efficiency and sequence coverage obtained using infrared multiphoton dissociation (IRMPD) in the Omnitrap; determine maximum efficiency as a ratio of the total fragment charge over precursor charge.
- testing using ubiquitin and cytochrome C molecular ions the efficiency and sequence coverage obtained using untravialet photodissociation (UV PD) in the Omnitrap; determine maximum efficiency as a ratio of the total fragment charge over precursor charge.

Task 2.3 Testing in situ ECD, HAB and CED MS/MS techniques (KI, FASM, IP, NTU, M15-19): Testing & optimization
- testing using ubiquitin and cytochrome C molecular ions the efficiency and sequence coverage obtained using electron capture dissociation (ECD) in the Omnitrap; determine maximum efficiency as a ratio of the total fragment charge over precursor charge. Optimization of experimental parameters to achieve maximum possible efficiency, determination of minimum time needed to perform.
- testing using ubiquitin and cytochrome C molecular ions the efficiency and sequence coverage obtained using hydrogen atom dissociation (HAD) in the Omnitrap; determine maximum efficiency as a ratio of the total fragment charge over precursor charge. Optimization of experimental parameters to achieve maximum possible efficiency, determination of minimum time needed to perform.
- testing using ubiquitin and cytochrome C molecular ions the efficiency and sequence coverage obtained using Coulomb explosion dissociation (CED) in the Omnitrap; determine maximum efficiency as a ratio of the total fragment charge over precursor charge. Optimization of experimental parameters to achieve maximum possible efficiency, determination of minimum time needed to perform.

Task 2.4 Application of ECD, HAB and CED MS/MS techniques to analysis of proteins (KI, TF, M16-23)
- Using ECD MS/MS in the Omnitrap, analyze mixture of soluble proteins from E. coli in the online LC-MS/MS regime; determine the number of proteins detected, average sequence coverage and average search engine score in protein identification.
- Using HAD MS/MS in the Omnitrap, analyze mixture of soluble proteins from E. coli in the online LC-MS/MS regime; determine the number of proteins detected, average sequence coverage and average search engine score in protein identification.
- Using HAD MS/MS in the Omnitrap, analyze mixture of soluble proteins from E. coli in the online LC-MS/MS regime; determine the number of proteins detected, average sequence coverage and average search engine score in protein identification.

Participation per Partner

Partner number and short name	WP2 effort
1 - KI	19.00
2 - FASMATECH SA	4.00
3 - THERMO FISHER	3.00
6 - TNTU	8.00
7 - IP	8.00
Total	42.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D2.1	in situ testing of the optimized CAD MS/MS protocol	1 - KI	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	18
D2.2	Protocol of in situ testing of the optimized CAD	1 - KI	Report	Confidential, only for members of the consortium (including the Commission Services)	20
D2.3	in situ testing optimized ECD, HECD and EID MS/MS	1 - KI	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	30

Description of deliverables

D2.1: Protocol of in situ testing of the optimized ion isolation technique
D2.2: Protocol of in situ testing of the optimized CAD MS/MS
D2.3: Protocol of in situ testing of the optimized ECD, HECD and EID MS/MS

D2.1 : in situ testing of the optimized CAD MS/MS protocol [18]

Protocol of in situ testing of the optimized ion isolation technique

D2.2 : Protocol of in situ testing of the optimized CAD [20]

Protocol of in situ testing of the optimized CAD MS/MS

D2.3 : in situ testing optimized ECD, HECD and EID MS/MS [30]

Protocol of in situ testing of the optimized ECD, HECD and EID MS/MS

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification

Work package number [9]	WP3	Lead beneficiary [10]	2 - FASMATECH SA
Work package title	Development and application of H-atom bombardment (HAB) MS/MS techniques		
Start month	1	End month	30

Objectives

Development and application of a novel hydrogen atom source with hyper-thermal energies.

Description of work and role of partners

WP3 - Development and application of H-atom bombardment (HAB) MS/MS techniques [Months: 1-30]
FASMATECH SA, KI, THERMO FISHER , TNTU, IP
Task 3.1 Designing the hyper-thermal H-atom gun (FASM, KI, IP, NTU, TF, M1- M7). The design will be based on a pulse plasma source utilizing DC potentials and fast pulse valves for raising pressure above the breakdown limit. Ion pulse transients of the order of a few tens of ms will be generated which will contain atomic ions of hydrogen. These ions will be thermalized via interactions with highly polished surfaces positioned coaxially with the expansion axis of the pulsed plasma beams.
Task 3.2 Building the hyper-thermal H-atom gun (FASM, KI, NTU, IP, TF, M6- M11)
The hyperthermal plasma gun will be developed and tested on a separate vacuum chamber for iterating the design and optimizing conditions of H atom production. Measurements will be performed with a residual gas analyzer to monitor reaction products in the plasma jet. Stopping curve experiments will also be performed for measuring the kinetic energy spread of the pulsed ion and neutral beams ejected from the plasma source. Critical parameters such as maintaining high vacuum conditions outside the source will also be evaluated carefully so as to not affect the ultra-high vacuum conditions for the orbitrap mass analyzer to operate smoothly.
Task 3.3 Interfacing the H-atom gun system with Omnitrap (FASM, KI, NTU, IP, TF, M11- 12)
Critical parameters such as maintaining high vacuum conditions outside the source will also be evaluated carefully so as to not affect the ultra-high vacuum conditions for the orbitrap mass analyzer to operate smoothly. Mechanical design considerations will also be critical for fitting the gun due to restrictions in the QE instrument design. Additional pumping capabilities will be employed if necessary.
Task 3.4 Develop of software for HAB MS/MS-Omnitrap combination (FASM, KI, NTU, IP, TF, M8-13)
The software will include additional instructions available in the instruction list of the omnitrap software to allow the user to define parameters such as the number of gas pulses applied sequentially, the amount of gas released in the system, the energy of the beam and other conditions that will permit optimization of protein ion fragmentation reactions. These will be part of a bundle instruction that can be introduced in the sequence list of any experiment performed in the omnitrap.
Task 3.5 Testing the HAB MS/MS- Omnitrap combination (FASM, KI, NTU, IP, TF, M11- 13)
After the successful completion of the hyperthermal H atom gun on the stand-alone vacuum chamber, tests will include ESI of proteins using the QE platform and testing H attachment and fragmentation reactions using standard protein ions. Optimization tests will be performed and energy-resolved experiments will be carried out to investigate the associated gas phase reaction effects.
Task 3.6 Optimizing the software and hardware for HAB MS/MS. (FASM, KI, TF M18- M22)
Feedback from the users in KI and IP will be essential for improvng the technology. This feedback will be provided after exhasutive experiments on the two prototype units for a given set of proteins and understanding of the gas phase mechanism responisble for fragmentation.
Task 3.7 HAB MS/MS analysis of mAbs. (FASM, KI, M18- M24)
The reactions of H atom produced from plasma jets will be tested on mAbs and the effects will be investigated using advanced sequence algorithms for deciphering fragmentation patterns and measuring sequence coverage. H attachment reactions will also be evaluated and used for deciphering between even and odd electron species. The complementarity of this technique to ECD, CID or other standard fragmentation techniques will be evaluated.

Participation per Partner

Partner number and short name	WP3 effort
1 - KI	37.00

Partner number and short name	WP3 effort
2 - FASMATECH SA	33.00
3 - THERMO FISHER	1.00
6 - TNTU	7.00
7 - IP	7.00
Total	85.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D3.1	Prototype of the HAB gun installed and tested protocol	2 - FASMATECH SA	Other	Confidential, only for members of the consortium (including the Commission Services)	15
D3.2	Optimized HAB guns installed and tested - protocols	2 - FASMATECH SA	Other	Confidential, only for members of the consortium (including the Commission Services)	30

Description of deliverables

D3.1. One prototype of the HAB gun installed and tested at KI – protocol of test
D3.2. Two tested, optimized HAB guns installed and tested at KI, IP– protocols of tests

D3.1 : Prototype of the HAB gun installed and tested protocol [15]

One prototype of the HAB gun installed and tested at KI – protocol of test

D3.2 : Optimized HAB guns installed and tested - protocols [30]

Two tested, optimized HAB guns installed and tested at KI, IP– protocols of tests

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS7	Suffic HAB MS/MS demonstrated	2 - FASMATECH SA	29	HAB MS/MS spectra of proteins demonstrated

Work package number [9]	WP4	Lead beneficiary [10]	1 - KI
Work package title	Development and application of Coulomb explosion MS/MS technique		
Start month	18	End month	39

Objectives

To develop the novel techniques utilizing 100-1000 eV electrons to achieve Coulomb explosion.

Description of work and role of partners

WP4 - Development and application of Coulomb explosion MS/MS technique [Months: 18-39]
KI, FASMATECH SA, Biomotif AB, TNTU, IP
Task 4.1 Designing the 100-1000 eV pulsed electron source (FASM, KI, NTU, IP, BM; M18 – M20).
First, it is important to choose the source of electrons (electron gun; cathode). The challenge is to have a combination of high electron current, low energy spread and high capacity to withstand poisoning by chemically active contaminants in the background gas. Among the candidates are Ta and W directly heated cathodes, BaO dispenser cathodes and field-emission cathodes.
The second challenge is to design the heating electronics that can be biased by several hundred volts, up to -1000 V. Despite the bias, the voltage on the emitting surface has to be stable within ±0.1 V.
The thirds challenge is to design pulsing electronics that would normally prevent the electrons from coming out of the source, that is to provide at least a hundred volts more negative potential than the bias, and then during the pulsing event to rapidly open up the gate, by pulsing a voltage by at least a hundred volts more positive than the bias.
The fourth challenge is to design the mechanical part of the source, capable of withstanding the high temperature of the electron source, as well as absorb stray electrons without charging up.
The last but not least, vacuum system should provide high vacuum in the source and rapid pump-down at the conditions of high temperature.

Task 4.2 Building the 100-1000 eV pulsed electron source. (FASM, KI, NTU, IP, BM; M20 – M22)
Building the pulsed electron source includes the following steps:
- manufacturing or acquiring the electrical components;
- manufacturing or acquiring the mechanical components;
- manufacturing or acquiring the vacuum components;
- acquiring the electron gun (cathode);
- assembling the parts;
- testing the operation in the autonomic regime.

Task 4.3 Interfacing the 100-1000 eV pulsed electron source with Orbitrap MS (FASM, KI, NTU, IP, BM; M22 -23)
Interfacing the pulsed electron source with the Orbitrap MS involves the following steps:
- obtaining a programmed trigger signal from the Orbitrap MS;
- interfacing the programmed trigger with the electronics of the electron source;
- programming the trigger during an MS/MS event in the Omnitrap;
- testing the combined performance of the pulsed electron source with the Orbitrap MS.

Task 4.4 Testing the Coulomb explosion MS/MS. (Lead: KI, Part: FASM, NTU, BM, IP; M23 – M24)
- performing Electron Ionization Dissociation (EID) MS/MS experiments on ubiquitin and cytochrome C ions, with electron energy spanning the range of 10 to 1000 eV;
- measuring the cross sections of ionization and fragmentation in EID, as well as charge states of the fragments;
- testing the hypothesis that the cross section for ionization and/or certain fragmentation channels will smoothly change around 300-400 eV, indicating the onset of the Coulomb explosion mechanism.

Task 4.5 Optimizing the hardware and software for Coulomb explosion MS/MS. (FASM, KI, NTU, IP; M23-25)
Once detected, the Coulomb explosion process can be optimized by changing the following parameters:
- electron energy;
- parameters of the trapping voltages in the Omnitrap – biases, frequency, amplitude;
- electron current;
- pulse duration;
- ion cooling after the MS/MS event: gas, pressure, duration,

to maximize the fragment ion yield and achieve >100% fragmentation efficiency (i.e., ion fragment charge exceeding the precursor ion charge).

Task 4.6 Application of Coulomb explosion dissociation (CED) MS/MS to mAbs. (KI, FASM, NTU, IP; M23-28)
Once optimized, the Coulomb explosion MS/MS will be applied to mAbs, with attention to be paid to:
- fragment ion yield;
- cleavages of disulfide bonds;
- cleavages of N-Ca bonds;
- other backbone cleavages;
- side chain cleavages;
- fragmentation efficiency;
- sequence coverage.

Participation per Partner

Partner number and short name	WP4 effort
1 - KI	26.00
2 - FASMATECH SA	13.00
5 - Biomotif AB	2.00
6 - TNTU	4.00
7 - IP	4.00
Total	49.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D4.1	Protocol- CED gun prototype installed and tested	1 - KI	Other	Confidential, only for members of the consortium (including the Commission Services)	30
D4.2	Protocols: CED guns installed and tested	1 - KI	Other	Confidential, only for members of the consortium (including the Commission Services)	39

Description of deliverables

D4.1. One prototype of the CED gun installed and tested at KI – protocol of test
D4.2. Two tested, optimized CED guns installed and tested at KI and IP – protocols of tests

D4.1 : Protocol- CED gun prototype installed and tested [30]

One prototype of the CED gun installed and tested at KI – protocol of test.

D4.2 : Protocols: CED guns installed and tested [39]

Two tested, optimized CED guns installed and tested at KI and IP – protocols of tests

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS12	Development of CED MS/MS	1 - KI	42	CEB MS/MS spectra of proteins

Work package number [9]	WP5	Lead beneficiary [10]	5 - Biomotif AB
Work package title	Development of pI-Trap-ESI combination		
Start month	15	End month	42

Objectives

Modify the pI-Trap and build an ESI interface for effective clean-up, fractionation, and ionization of Abs.

Description of work and role of partners

WP5 - Development of pI-Trap-ESI combination [Months: 15-42]
Biomotif AB, KI, THERMO FISHER , TNTU, IP, MS VISION
Task 5.1 Designing and testing the pI-cell optimized for large proteins. (BM, KI, MS, TF; M15- M18)
The peek capillary tubing and the tubular nafion membranes must be optimized in terms of length and diameter for the best performance analyzing large proteins. We will also work with different ampholytes for getting optimum performance for protein analysis. Advice and expertise will be collected from partners KI, MS and TF.
Task 5.2 Design and testing buffer exchanger ESI interface for pI-Trap. (BM, MS, TF; M16- M18)
The micro dialysis membrane and the inner of outer tubing must be optimized for best performance when analyzing large proteins. The best volume needed will also be investigated as well as optimum time interval for the buffer exchange process. Work will be done with valuable input from MS and TF.
Task 5.3 Design controlling software for pI-Trap-Orbitrap combination (BM, TF, MS, NTU; M18- 20)
This will be done in close collaboration with TF, MS and NTU. We will also need support and input from Spark Holland and DataApex for best adaptation with the software controlling autosampler and fraction collector.
Task 5.4 Testing the pI-Trap-Orbitrap combination for proteins (BM, KI, TF, IP; M20- 24)
This task is best done in conjunction with partners KI, IP and TF. The testing will take place at the Karolinska Institutet on samples from IP and KI.

Participation per Partner

Partner number and short name	WP5 effort
1 - KI	15.00
3 - THERMO FISHER	3.00
5 - Biomotif AB	17.00
6 - TNTU	2.00
7 - IP	2.00
8 - MS VISION	3.00
Total	42.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D5.1	Prototype pI-Trap-ESI installed and tested– protocol	5 - Biomotif AB	Other	Public	20

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D5.2	Two tested, optimized pI-Trap-ESI installed and tested	5 - Biomotif AB	Other	Public	42

Description of deliverables

D5.1. One prototype of the pI-Trap-ESI installed and tested at KI – protocol of test
D5.2. Two tested, optimized pI-Trap-ESI installed / tested at KI and IP – protocols of tests

D5.1 : Prototype pI-Trap-ESI installed and tested– protocol [20]

One prototype of the pI-Trap-ESI installed and tested at KI – protocol of test

D5.2 : Two tested, optimized pI-Trap-ESI installed and tested [42]

Two tested, optimized CED guns installed and tested at KI and IP – protocols of tests

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS8	Interfacing pI-Trap-Orbitrap	5 - Biomotif AB	32	Excellent fractionation of mAb isoforms and ionization
MS13	All technologies interfaced	5 - Biomotif AB	42	Sequencing mAb

Work package number [9]	WP6	Lead beneficiary [10]	3 - THERMO FISHER
Work package title	Modification of the Orbitrap mass spectrometer		
Start month	2	End month	42

Objectives

To improve the performance of the Orbitrap Q Exactive HF X mass spectrometer for top-down MS/MS of Abs.

Description of work and role of partners

WP6 - Modification of the Orbitrap mass spectrometer [Months: 2-42]
THERMO FISHER , KI
Task 6.1. Installation of a loaned Q Exactive instrument to Fasmatech to support Omnitrap development
A Q Exactive HF instrument will be configured to be interfaced to Omnitrap by removing the charge detector on the back of the HCD cell and adding functionality of ion transfer to and from the Omnitrap. Once the instrument is shipped and installed at Fasmatech, dedicated trigger signals will be provided to initiate the operational sequence of the Omnitrap and software training and support provided to Fasmatecch and Spectroswiss in order to fulfil corresponding tasks of the project. This instrument will be focused on optimizing Omnitrap functionalities.

Task 6.2. Modified Orbitrap Q Exactive HF X delivered and installed at KI - protocol
In parallel to Task 6.1, a standard Orbitrap Q Exactive HF-X (or a similar high-end instrument) will be modified to improve its performance for desolvation and transmission of intact antibodies. Based on research using Q Exactive UHMR and standard HF-X instruments, there is clearly a reserve for optimizing the desolvation region of the atmosphere-to-vacuum interface that deserves a more detailed exploration.
In parallel to this, a joint work with Spectroswiss and Fasmatech will be started on integration of instrument control software using application programming interface (API) to be provided by TF. This work includes also development of tuning and calibration procedures specific for antibody analysis in order to ensure best top-down performance, integration of data for all fragmentation methods and cross-section measurements.
After testing of all functional units, the resulting will be delivered and installed at KI and performance protocol will be completed for a test set of compounds.

Participation per Partner

Partner number and short name	WP6 effort
1 - KI	10.00
3 - THERMO FISHER	28.00
Total	38.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D6.1	Installation of Q Exactive instrument for Omnitrap development	3 - THERMO FISHER	Other	Confidential, only for members of the consortium (including the Commission Services)	12

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D6.2	Modified Orbitrap Q Exactive HF X installed	3 - THERMO FISHER	Other	Public	42

Description of deliverables

Deliverables:
D6.1. Installation of a loaned Q Exactive instrument to Fasmatech to support Omnitrap development
D6.2. Modified Orbitrap Q Exactive HF X delivered and installed at KI - protocol

D6.1 : Installation of Q Exactive instrument for Omnitrap development [12]

Installation of a loaned Q Exactive instrument to Fasmatech to support Omnitrap development

D6.2 : Modified Orbitrap Q Exactive HF X installed [42]

Modified Orbitrap Q Exactive HF X delivered and installed at KI - protocol

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS3	Installation of Q Exactive instrument for Omnitrap development	3 - THERMO FISHER	12	Installation of a loaned Q Exactive instrument to Fasmatech to support Omnitrap development
MS9	Interfacing pI-Trap-Orbitrap OMNI-ORBI combination	2 - FASMATECH SA	32	Capacity to sequence intact proteins

Work package number [9]	WP7	**Lead beneficiary** [10]	4 - SPECTROSWISS
Work package title	Signal detection and data processing		
Start month	1	**End month**	40

Objectives

To design, develop, test and optimize FTMS Booster for top-down analysis of large proteins.
To develop and implement algorithms for data processing and analysis for top-down of large proteins.

Description of work and role of partners

WP7 - Signal detection and data processing [Months: 1-40]
SPECTROSWISS, KI, FASMATECH SA, TNTU, IP
Task 7.1 Develop data acquisition system (FTMS Booster) for protein top-down analysis (SPS: the lead, performs the task using SPS infrastructure and personnel, M1-M14)
Task 7.2 Develop a transient-based decay constant deconvolution approach (SPS: the lead, supervision and main development, TNTU: support for related data analysis software development, KI: support for fundamentals and vision of the approach development and applications, M4-M12)
Task 7.3 Develop data processing software for protein top-down analysis (SPS: the lead, development and implementation of time-domain data (transients) processing aiming for protein analysis; TNTU: support for related data analysis software development, KI and IP: software specifications formulation, software evaluation, M1-M18)
Task 7.4 Evaluating the FTMS Boosters in protein top-down analysis in a laboratory environment. (SPS: the lead, supervision and support of the evaluation procedure, optimization and facilitation of FTMS Booster connectivity to the Orbitrap platforms on-site at KI and IP, troubleshooting; KI and IP: perform experimental evaluation of the FTMS Boosters in their laboratories, M19-M34)
Task 7.5 Development of the top-down data analysis software optimized for Abs (TNTU: the lead, SPS: support for related data analysis software development, interfacing data processing and data analysis software architectures and tools, FASM, KI and IP: software specifications formulation, and software evaluation, M1-M29)

Participation per Partner

Partner number and short name	WP7 effort
1 - KI	35.00
2 - FASMATECH SA	1.00
4 - SPECTROSWISS	44.00
6 - TNTU	21.00
7 - IP	7.00
Total	108.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D7.1	Two Prototype FTMS Booster installed and tested-protocol	4 - SPECTROSWISS	Other	Confidential, only for members of the consortium (including the Commission Services)	30

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D7.2	Top-down analysis software	4 - SPECTROSWISS	Other	Confidential, only for members of the consortium (including the Commission Services)	35
D7.3	Optimized FTMS Boosters test protocols	4 - SPECTROSWISS	Other	Confidential, only for members of the consortium (including the Commission Services)	40

Description of deliverables

D7.1. Protocol of installation and testing a prototype FTMS Booster at KI
D7.2. Protocols of installation and testing of two optimized FTMS Boosters at KI and IP
D.7.3. Top-down data processing and analysis software

D7.1 : Two Prototype FTMS Booster installed and tested-protocol [30]

Protocol of installation and testing a prototype FTMS Booster at KI

D7.2 : Top-down analysis software [35]

Top-down data processing and analysis software distributed to participants. Includes implementation of an approach to mass spectra deconvolution via transient decay rates.

D7.3 : Optimized FTMS Boosters test protocols [40]

Protocols of installation and testing of two optimized FTMS Boosters at KI and IP

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS1	Demonstrated effectiveness of product ion isotopic distribution deconvolution	4 - SPECTROSWISS	12	Demonstrated effectiveness of product ion isotopic distribution deconvolution in simulated and reference experimental top-down mass spectra via time-domain data (transient) damping approach.
MS6	Data processing algorithms and software for simulated and experimental top-down mass spectra and time-domain data transients	4 - SPECTROSWISS	18	Data processing algorithms and software are produced and tested on simulated and experimental top-down mass spectra and time-domain data (transients) for top-down data analysis, including approaches for big data analysis of full profile mass spectra for maximizing sensitivity and dynamic range

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS10	Data analysis algorithms and software for simulated and experimental top-down data analysis	6 - TNTU	35	Data analysis algorithms and software are produced and tested on simulated and experimental top-down mass spectra for top-down data analysis, including approaches to product ion assignment with and without mass spectra
MS11	Two FTMS Booster prototypes are designed, implemented, and evaluated	4 - SPECTROSWISS	40	Two FTMS Booster prototypes are designed, implemented, and evaluated at Spectroswiss for enabling in-line digital signal processing capable of on-the-fly delivering mass spectra

Work package number [9]	WP8	Lead beneficiary [10]	8 - MS VISION
Work package title	Dissemination, Communication & Exploitation		
Start month	3	End month	42

Objectives

To develop an exploitation and communication plan to ensure successful uptake for the TopSpec technologies. To integrate all technologies developed in work packages 1-7 into a TopSpec platform.
To apply the integrated TopSpec platform to model and real-life applications for Abs analysis.

Description of work and role of partners

WP8 - Dissemination, Communication & Exploitation [Months: 3-42]
MS VISION, KI, FASMATECH SA, THERMO FISHER , SPECTROSWISS, Biomotif AB, TNTU, IP
Task 8.1 Dissemination activities. M1-M36.
- Creating and publishing the public dissemination material (Website, posters, brochures, videos),
- Adapting the dissemination support to the target,
- keeping track of publications and public disclosures by creating a database.

Task 8.2 Knowledge Management and IPR. M1-M36
- Management of the pre-existing knowledge needed to achieve the work (background), the knowledge created during the project (foreground), and the knowledge created in parallel to the project (side-ground) by either partners or other parties that might impact the project.

Task 8.3 Exploitation strategy of the results M1-M36
- Identification of the internal and external stockholders.
- Determining the synergies between them to integrate the results, identify the weak points, assess the usability of the results.
- Identify the competing technical approaches
- Analyze the evolving socio-economic context including user needs, overall market trends.

Task 8.4 Demonstration workshop M22
- The stakeholders identified and presented in 8.3 will be invited to analyze project results in order to evaluate the scientific relevance, performance and transferability of the technology.

Task 8.5 Management of patent strategy and freedom to operate (FTO), M1-36.

- Develop an IP protection strategy at the start of the project (M3).
- Monitor that the newly created IP falls under the Consortium Agreement.

Task 8.6 Public engagement, M1-36.
- Create articles with easy public access through project website
- Publish popular articles in general science magazines
- Giving interviews to news reporters (newspapers, TV, radio etc.)

Task 8.7 Develop and implement a common business strategy for market introduction. M12-36.
- Develop a common business strategy for market introduction through consultations within Consortium.
- Implement the developed business strategy for market introduction.

Task 8.9 Organizing relevant conferences: M6-36.
- Organizing a conference of the UppCon series (Uppsala conference on Electron Capture Dissociation and related phenomena, run since 2003).
- Organizing a conference on Top-down analysis of proteins;
- Organizing a summer school on Electron Capture Dissociation and related phenomena Top-down analysis of proteins, as part of the annual MSBM (MS in biotechnology and medicine) summer school in Dubrovnik, Croatia.
- Organizing hands-on course will be arranged at KI, and will be open to European students.

Task 8.10 Communication to commercial research organizations. M12-36.
- As we anticipate significant interest in TopSpec from the Pharma industry, we will act through technical media channels, B2B, fairs and conferences.

Participation per Partner

Partner number and short name	WP8 effort
1 - KI	19.00
2 - FASMATECH SA	3.00
3 - THERMO FISHER	5.00
4 - SPECTROSWISS	11.00
5 - Biomotif AB	5.00
6 - TNTU	9.00
7 - IP	9.00
8 - MS VISION	10.00
Total	71.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D8.1	IP protection strategy finalized	8 - MS VISION	Other	Public	3
D8.2	Draft Exploitation plan and Business strategy document	8 - MS VISION	Report	Public	12
D8.3	Young scientist TopSpec technology workshop	8 - MS VISION	Other	Public	39
D8.4	Public demonstrations of TopSpec technology	8 - MS VISION	Report	Public	39
D8.5	Scientific reports and publications	8 - MS VISION	Report	Public	42
D8.6	Exploitation plan and Business strategy document	8 - MS VISION	Report	Confidential, only for members of the consortium (including the Commission Services)	42

Description of deliverables

D8.1 IP protection strategy finalized
D8,2 Draft of Exploitation Plan and Buisiness strategy document delivered
D8.3 Completion of Young scientist TopSpec technology workshop
D8.4 Public demonstrations of TopSpec technology
D8.5 Scientific reports and publications
D8.6 Exploitation plan and Business strategy document published

D8.1 : IP protection strategy finalized [3]
IP protection strategy finalized

D8.2 : Draft Exploitation plan and Business strategy document [12]
Draft plan of Exploitation plan and Business strategy document delivered

D8.3 : Young scientist TopSpec technology workshop [39]
Completion of Young scientist TopSpec technology workshop

D8.4 : Public demonstrations of TopSpec technology [39]
Public demonstrations of TopSpec technology

D8.5 : Scientific reports and publications [42]
Scientific reports and publications delivered

D8.6 : Exploitation plan and Business strategy document [42]
Exploitation plan and Business strategy document published

Schedule of relevant Milestones

Milestone number[18]	**Milestone title**	**Lead beneficiary**	**Due Date (in months)**	**Means of verification**

Work package number [9]	WP9	Lead beneficiary [10]	1 - KI
Work package title	Project Management and Administration		
Start month	1	End month	42

Objectives

To manage the project, partners, project tasks and activities through effective organization and administration.

Description of work and role of partners

WP9 - Project Management and Administration [Months: 1-42]
KI, FASMATECH SA, THERMO FISHER , SPECTROSWISS, Biomotif AB, TNTU, IP, MS VISION
Task 9.1 Design and publish the TopSpec project website. (KI, All: M1-2).
- Appoint the web master;
- Choose the web page design;
- Create the first edition of the web site;
- Update regularly the web site and publish news.

Task 9.2 Preparation and delivery of a data management plan. (KI; All: M4-6)
- Preparation of a data management plan, based on the EU recent rules and regulations.
- Delivery of the data management plan.

Task 9.3 Project management and administration (KI: M1-36).
- The KI administration will ensure all legal agreements are in place before start.
- Organize and document consortium and steering group meetings (every 6 months at partner sites).

Task 9.4 Financial Control and management (KI: M1-36). A financial manager at the KI will be hired.

Participation per Partner

Partner number and short name	WP9 effort
1 - KI	30.00
2 - FASMATECH SA	6.00
3 - THERMO FISHER	6.00
4 - SPECTROSWISS	6.00
5 - Biomotif AB	6.00
6 - TNTU	6.00
7 - IP	6.00
8 - MS VISION	6.00
Total	72.00

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D9.1	Logo and Website launch and public accessibility	1 - KI	Other	Public	2

List of deliverables

Deliverable Number[14]	Deliverable Title	Lead beneficiary	Type[15]	Dissemination level[16]	Due Date (in months)[17]
D9.2	Data management plan	1 - KI	ORDP: Open Research Data Pilot	Public	6
D9.3	Technical/scientific review meeting documents	1 - KI	Report	Confidential, only for members of the consortium (including the Commission Services)	13
D9.4	Scientific review meeting documents	1 - KI	Report	Confidential, only for members of the consortium (including the Commission Services)	30
D9.5	Final: Technical/ scientific review meeting documents	1 - KI	Report	Confidential, only for members of the consortium (including the Commission Services)	42

Description of deliverables

D9.1 Project logo and website launch and public accessibility. Site will be constantly maintained and updated.
D9.2 Data management plan will be presented.
D9.3 Technical/Scientific review meeting documents will be provided.
D9.4 Final review meetings of the project.

D9.1 : Logo and Website launch and public accessibility [2]

Project logo and website launch and public accessibility. Site will be constantly maintained and updated.

D9.2 : Data management plan [6]

Data management plan

D9.3 : Technical/scientific review meeting documents [13]

Delivered draft agenda and presentations during review meeting following RP1.

D9.4 : Scientific review meeting documents [30]

Delivered draft agenda and presentations of the review meeting.

D9.5 : Final: Technical/scientific review meeting documents [42]

Delivered draft agenda and presentations of the review meeting following the 2nd reporting period

Schedule of relevant Milestones

Milestone number[18]	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification

1.3.4. WT4 List of milestones

Milestone number[18]	Milestone title	WP number[9]	Lead beneficiary	Due Date (in months)[17]	Means of verification
MS1	Demonstrated effectiveness of product ion isotopic distribution deconvolution	WP7	4 - SPECTROSWISS	12	Demonstrated effectiveness of product ion isotopic distribution deconvolution in simulated and reference experimental top-down mass spectra via time-domain data (transient) damping approach.
MS2	Omnitraps & IMS Electronics design	WP1	2 - FASMATECH SA	12	Omnitraps & IMS Electronics design finalized
MS3	Installation of Q Exactive instrument for Omnitrap development	WP6	3 - THERMO FISHER	12	Installation of a loaned Q Exactive instrument to Fasmatech to support Omnitrap development
MS4	Omnitraps & IMS P.O.s sent to suppliers	WP1	2 - FASMATECH SA	12	Omnitraps & IMS P.O.s sent to suppliers
MS5	Omnitraps & IMS Mechanical design	WP1	2 - FASMATECH SA	12	Omnitraps & IMS Mechanical design finalised
MS6	Data processing algorithms and software for simulated and experimental top-down mass spectra and time-domain data transients	WP7	4 - SPECTROSWISS	18	Data processing algorithms and software are produced and tested on simulated and experimental top-down mass spectra and time-domain data (transients) for top-down data analysis, including approaches for big data analysis of full profile mass spectra for maximizing sensitivity and dynamic range
MS7	Suffic HAB MS/MS demonstrated	WP3	2 - FASMATECH SA	29	HAB MS/MS spectra of proteins demonstrated
MS8	Interfacing pI-Trap-Orbitrap	WP5	5 - Biomotif AB	32	Excellent fractionation of mAb isoforms and ionization
MS9	Interfacing pI-Trap-Orbitrap OMNI-ORBI combination	WP1, WP6	2 - FASMATECH SA	32	Capacity to sequence intact proteins
MS10	Data analysis algorithms and software for simulated and experimental top-down data analysis	WP7	6 - TNTU	35	Data analysis algorithms and software are produced and tested on simulated and experimental top-down mass spectra for top-down data analysis, including approaches to product ion assignment with and without mass spectra
MS11	Two FTMS Booster prototypes are	WP7	4 - SPECTROSWISS	40	Two FTMS Booster prototypes are designed, implemented, and evaluated

Milestone number[18]	Milestone title	WP number[9]	Lead beneficiary	Due Date (in months)[17]	Means of verification
	designed, implemented, and evaluated				at Spectroswiss for enabling in-line digital signal processing capable of on-the-fly delivering mass spectra
MS12	Development of CED MS/MS	WP4	1 - KI	42	CEB MS/MS spectra of proteins
MS13	All technologies interfaced	WP5	5 - Biomotif AB	42	Sequencing mAb

1.3.5. WT5 Critical Implementation risks and mitigation actions

Risk number	Description of risk	WP Number	Proposed risk-mitigation measures
1	Low efficiency of HAB MS/MS (high)	WP3	Redesign the HAB gun, increase the H-atom flux and their energy
2	Low efficiency of CEB MS/MS (high)	WP4	Redesign the 100-1000 eV electron gun and/or optics
3	Delay in software design (medium)	WP7	Additionally, employ professional programmers
4	Despite MS/MS efforts, full sequence coverage of mAb is not obtained (high)	WP3, WP4, WP7	Increase charge state of precursor ions by super-charging via buffer exchange. Use multiple fill technique. Add IR and/or UV laser.

1.3.6. WT6 Summary of project effort in person-months

	WP1	WP2	WP3	WP4	WP5	WP6	WP7	WP8	WP9	Total Person/Months per Participant
1 - KI	0	19	37	26	15	10	35	19	30	191
2 - FASMATECH SA	39	4	33	13	0	0	1	3	6	99
3 - THERMO FISHER	3	3	1	0	3	28	0	5	6	49
4 - SPECTROSWISS	0	0	0	0	0	0	44	11	6	61
5 - Biomotif AB	0	0	0	2	17	0	0	5	6	30
6 - TNTU	0	8	7	4	2	0	21	9	6	57
7 - IP	0	8	7	4	2	0	7	9	6	43
8 - MS VISION	0	0	0	0	3	0	0	10	6	19
Total Person/Months	42	42	85	49	42	38	108	71	72	549

1.3.7. WT7 Tentative schedule of project reviews

Review number [19]	Tentative timing	Planned venue of review	Comments, if any
RV1	14	Brussels	If necessary the location might be different
RV2	31	Brussels	TBD
RV3	43	Brussels	TBD

1. Project number

The project number has been assigned by the Commission as the unique identifier for your project. It cannot be changed. The project number **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

2. Project acronym

Use the project acronym as given in the submitted proposal. It can generally not be changed. The same acronym **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

3. Project title

Use the title (preferably no longer than 200 characters) as indicated in the submitted proposal. Minor corrections are possible if agreed during the preparation of the grant agreement.

4. Starting date

Unless a specific (fixed) starting date is duly justified and agreed upon during the preparation of the Grant Agreement, the project will start on the first day of the month following the entry into force of the Grant Agreement (NB : entry into force = signature by the Agency). Please note that if a fixed starting date is used, you will be required to provide a written justification.

5. Duration

Insert the duration of the project in full months.

6. Call (part) identifier

The Call (part) identifier is the reference number given in the call or part of the call you were addressing, as indicated in the publication of the call in the Official Journal of the European Union. You have to use the identifier given by the Commission in the letter inviting to prepare the grant agreement.

7. Abstract

8. Project Entry Month

The month at which the participant joined the consortium, month 1 marking the start date of the project, and all other start dates being relative to this start date.

9. Work Package number

Work package number: WP1, WP2, WP3, ..., WPn

10. Lead beneficiary

This must be one of the beneficiaries in the grant (not a third party) - Number of the beneficiary leading the work in this work package

11. Person-months per work package

The total number of person-months allocated to each work package.

12. Start month

Relative start date for the work in the specific work packages, month 1 marking the start date of the project, and all other start dates being relative to this start date.

13. End month

Relative end date, month 1 marking the start date of the project, and all end dates being relative to this start date.

14. Deliverable number

Deliverable numbers: D1 - Dn

15. Type

Please indicate the type of the deliverable using one of the following codes:

R	Document, report
DEM	Demonstrator, pilot, prototype
DEC	Websites, patent fillings, videos, etc.
OTHER	
ETHICS	Ethics requirement
ORDP	Open Research Data Pilot
DATA	data sets, microdata, etc.

16. Dissemination level

Please indicate the dissemination level using one of the following codes:

PU	Public
CO	Confidential, only for members of the consortium (including the Commission Services)
EU-RES	Classified Information: RESTREINT UE (Commission Decision 2005/444/EC)
EU-CON	Classified Information: CONFIDENTIEL UE (Commission Decision 2005/444/EC)
EU-SEC	Classified Information: SECRET UE (Commission Decision 2005/444/EC)

17. Delivery date for Deliverable

Month in which the deliverables will be available, month 1 marking the start date of the project, and all delivery dates being relative to this start date.

18. Milestone number

Milestone number:MS1, MS2, ..., MSn

19. Review number

Review number: RV1, RV2, ..., RVn

20. Installation Number

Number progressively the installations of a same infrastructure. An installation is a part of an infrastructure that could be used independently from the rest.

21. Installation country

Code of the country where the installation is located or IO if the access provider (the beneficiary or linked third party) is an international organization, an ERIC or a similar legal entity.

22. Type of access

TA-uc	if trans-national access with access costs declared on the basis of unit cost,
TA-ac	if trans-national access with access costs declared as actual costs, and
TA-cb	if trans-national access with access costs declared as a combination of actual costs and costs on the basis of unit cost,
VA-uc	if virtual access with access costs declared on the basis of unit cost,
VA-ac	if virtual access with access costs declared as actual costs, and
VA-cb	if virtual access with access costs declared as a combination of actual costs and costs on the basis of unit cost.

23. Access costs

Cost of the access provided under the project. For virtual access fill only the second column. For trans-national access fill one of the two columns or both according to the way access costs are declared. Trans-national access costs on the basis of unit cost will result from the unit cost by the quantity of access to be provided.

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Topic: FET-Open research and innovation actions

History of changes

Changes	Section	Comment
Part A	Beneficiaries	THERMO FISHER SCIENTIFIC (BREMEN) GMBH: short name changed to THERMO FISHER BIOMOTIF AB: included address Solnavägen 9, 171 65, Stockholm Sweden SPECTROSWISS: included Department name and address Indirect costs have been edited manually according to 25% for all participants.
	General Information	Set to January 1st 2019 as requested by the Commission (Project Officer). **AMENDMENT 829157-12 (1 change):** Duration changed to 42 months
	Reporting Periods	Set at 12 month and 36 months. **AMENDMENT 829157-12 (1 change):** The reporting period at month 36 has been changed to month 42.
	Work Packages	Some of the WPs (WP2 (36), WP3 (24), WP5 (33), WP6 (36)) have been changed to match the final deliverable end months. As required by the Project officer we have included more information in the "Description of work and role of partners" sections. For WP7 we have changed the work effort of SPS to 44 (to reflect SPS changes in the budget). **AMENDMENT 829157-12 (9 changes):** The end months of WP1 (42), WP2 (30), WP3 (30), WP4 (39), WP5 (42), WP6 (42), WP7 (40), WP8 (42), WP9 (42) have been changed.
	Deliverables	The deliverables fore respective WP have (if needed) been changed so that they are in a sequential order. D2.1-3 had the Type "DO" which does not exist. Thus, D2.1 and D2.3 have been changed to "DEM" and D2.2 has been changed to "R". The leads in D4.1-2 as well as in D5.1-2 has been corrected (from proposal Table 3.1c) to match (more descriptive and correct Table 3.1a in the proposal). D6.2 has been changed to match the WP due date at M36. D9.1, "Logo and Website launch and public accessibility" has been changed to due date 2 months and with the insurance to be frequently updated as requested by the Project Officer. The ORDP data management plan is incorporated as deliverable D9.2, due M6. D9.3, "Technical/scientific review meeting documents", has been included as requested by the Project Officer with the due at M13. As requested, we have removed the deliverable "The financial and technical reports" and have included a new deliverable "Review meetings", D9.4 at M36. We have also included new deliverable D6.1, "Installation of Q Exactive instrument for Omnitrap development" at month 12, to meet the milestones requirements (at 12 month) as requested by the Project Officer. **AMENDMENT 829157-12 (18 changes):** The end months of deliverables D1.1 (28), D1.2 (39), D1.3(42), D2.3 (30), D3.2 (30), D4.1 (30), D4.2 (39), D5.2 (42), D6.2 (42), D7.1 (30), D7.2 (35), D7.3 (40), D8.3 (39), D8.4 (39), D8.5 (42), D8.6 (42) and D9.5 (42) have been changed. D9.4 (30), "Scientific review meeting documents" is new.
	Milestones	The milestones have (if needed) been changed so that they are in a sequential order. Several new milestones (M1-M5) have been included as suggested by the Project Officer to make sure that there are enough meaningful milestones at month 12 to enable a proper monitoring off project progress. Additionally milestones M6, M10 and M11 has been added. **AMENDMENT 829157-12 (7 changes):** The end months of the milestones M7 (29), M8 (32), M9 (32), M10 (35), M11 (40), M12 (42) and M13 (42), have been changed.
	Critical Risks	Critical Risks have been included in the same order as in the proposal.
Part B	1.1 Radical vision of a science-enabled technology	-
	1.2 Science-to-technology breakthrough that addresses this vision	-
	1.3 Interdisciplinarity and non-incrementality of the research proposed	-
	1.4 High risk, plausibility and flexibility of the research approach	**Page 8** How to improve the gender balance has been further addressed as requested**.** **New text is included:** new section*: "Biological samples: The risk associated with the translation of results into real biological samples is rather low, as we have access to antibodies related to diseases where the whole antibody repertoire is dominated by a small subset of antibodies and even by a single clone"*
	2.1 Impact on technology and society	-
	2.2 Impact on future leadership	-
	2.3 Measures for achieving impact	-
	3.1 Work plan and intermediate targets	**Page 10** WPs description and interactions has been changed as addressed by the Project Officer. **Page 11** A new **Gannt chart** has been included with the inclusion of new Deliverables and Milestones in sequential order. **AMENDMENT 829157-12 (2 changes). Page 11.** The Gantt chart and the Gantt chart text have been changed according to the changes made in Part A.
	3.2 Management structure, milestones and procedures	**Page 12** **New text is included:** *We plan that the Dissemination and Exploitation Plan will be developed by the end of the first Reporting Period (RP1, month 12) and that it will be updated for the subsequent Reporting Period at 36 months. Management procedures will*

		follow qualified majority. Consortium meetings will take place at least twice a year. Video conferences will take place every month. Ad hoc video conferences should be possible.
	3.3 Relevance of expertise in the consortium	**Page 112** **New text is included:** *Institute Pasteur (IP) is the historical cradle of the antibody research, and having them part of our consortium ensures that the experts are present. The fact that they are also experts in top-down sequencing of proteins is a perfect match of different expertise we need.*
	Table 3.4	Edits made according to issues addressed by the Project Officer (audit costs, more specific split of figures in the OGS justification section etc.). Note that in terms of the costs estimates it is difficult to provide a complete breakdown of everything that we will subsequently purchase together with its cost. This is research (we don't always know what we need until we have performed several trial and error steps). Furthermore the exchange rate is going through a spectacularly volatile period. Note that we have changed so that the reduction of the BM budget for 10 kEuro is suggested to be used to increase the SPS budget for "Other goods and services" by 8000 Euro. This will compensate for the audit costs (estimated 5000 Euro) which were originally estimated budget for consumables (10000 Euro) and to further strengthen it by (3000 Euro) to better serve the data sharing needs between the participants) of the project which will generate extremely large amounts of data.
	Part 4. 4.1 Participants	Part 4 included (pages 12-38) On page 24: Text is removed: On-campus access to a full range of Orbitrap FTMS instruments, including Q Exactive HF Orbitrap FTMS, and allied sample preparation infrastructure at the EPFL and University of Lausanne (UNIL) facilities. Reason: SPS ensures that sufficient infrastructure at the partner's site (SPS site) will be provided to develop and test the prototypes of he FTMS Boosters. KI personnel added on page 14. Thermo Fisher, San Jose, CA, has been removed on page 19.
	5.1 Ethics	Page 39. As requested, the ethical requirements are now addressed according to the instructions provided by the Project officer.
	5.2 Security	-
Budget Table		**SPS:** relocation of 60 kEuro from equipment budget to personnel (salary) budget. Justification: updated design suggested for the FTMS Booster prototypes will require more personnel work of SPS scientists for implementation of digital signal processing, which will be compensated by the reduction in the cost of the electronics components. The final result (the two FTMS Booster prototypes) will be more powerful and higher performance prototypes. Furthermore, the reduction of the BM budget for 10 kEuro is suggested to be used to increase the SPS budget for "Other Goods and Services" by 8000 Euro, which will compensate for the audit costs (estimated 5000 Euro) which were not originally included into SPS budget. This increase will allow SPS to restore the originally estimated budget for consumables (10000 Euro) and to further strengthen it (by 3000 Euro) to better serve the data sharing needs (between the participants) of the project which will generate extremely large amounts of data. **FASM:** Since Dr. Papanastasiou as Fasmatech's co-founder and R&D Director has not a direct employment with Fasmatech, his involvement in this project will be reported under the "SME owner without salary". In order to reflect Dr. Papanastasiou's involvement in this project we have put in 2580 hours in A4: "SME owners without salary", the system automatically calculates the applicable rate/hr (30.27), total unit cost= 78096.60. The amount of Employee cost was equally reduced (356400-78096.60= 278303.40). **Biomotif:** The budget is reduced with 10.000 Euro. We cannot justify to allocate valuable time for an EU audit based on only 5K Euro more.

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1. Excellence

1.1 Radical vision of a science-enabled technology

We envision the world where all diseases are cured by person's own immune system, which is the most complex and enigmatic part of the human organism. Indeed, in the blood of every human there are an astronomical number (10^{13}-10^{15}) of antibody (Ab) variants which differ in their amino acid (AA) sequences and post-translational modifications (PTMs). These Abs bind with high specificity to the antigens (Ag) of invaders such as viruses and other microorganisms, as well as to undesired modifications of the body´s own proteins. The bound Ab-Ag complex is then removed naturally from the body, which then upregulates (activates and increases) the production of the Abs. The latter, in turn, serve then as detectors of disease as well as treatment. The role of Abs-based drugs in modern medicine cannot be overestimated. Among the 10 most sold drugs half are Abs, with the total worldwide sales of $62 BN (QuintilesIMS, Dec 2016). These costs are a huge burden for EU health system, and their development is a major bottleneck in making the health system more efficient.

It has recently been found that when a common ailment affects different people, the AA sequences of Abs raised against the cause of the disease are often similar. Therefore, if it were possible to find the common sequence of Abs raised specifically at the beginning of a disease, it should be rather easy to produce a cure against that disease in the form of a therapeutic Ab with the same sequence. In fact, this is how the first therapeutic Abs were produced against Alzheimer's disease[1]. The general strategy would then be first to investigate the sequences of Abs that are upregulated in the blood of a group of early stage disease patients, then find the common sequences between these patients and finally produce and optimize an Ab with that sequence. For personalized treatment, the sequence of the person-specific Ab that is similar to the common patient sequence could be used. ***The bottleneck in this would-be strategy is the Ab sequencing itself.*** Genomics and transcriptomics are of little value here because of the poor connection between gene translation and the Ab expression levels. Recently however, we have found a way to tackle this problem using mass spectrometry (MS) of peptides (not whole Abs!) that we have named *SpotLight Proteomics*[2]. While this approach provides useful results, it leaves a huge knowledge gap, as it is hard to get to the sequence of the whole Ab (≈1400 AAs) from a known peptide 5-20 AA long. In principle, one can raise animal polyclonal Abs against the target peptide, and using them extract Abs containing this peptide from human blood. By fractionation, one can then identify which fraction has the highest specificity against the target peptide. But then the Ab in that fraction should be sequenced, which is the true bottleneck in this strategy. By digesting the Ab with several enzymes, one often obtains overlapping peptides, which, upon *de novo* sequencing by tandem MS, might align in a whole Ab sequence. But the risk of wrong or incomplete alignment in this bottom-up approach is very high, especially for variable regions. For verification, one needs to sequence the whole Ab and/or its structural subunits top-down, i.e., by fragmentation of the whole molecule in tandem MS as was pioneered by our team[3, 4, 5]. Now, here is where the major crux of problem is: ***Modern MS instrumentation can produce just 30-40% of Ab sequence coverage by top-down approach.***

1.2 Science-to-technology breakthrough that addresses this vision

[1] ND Prins, P Scheltens, Treating Alzheimer's disease with monoclonal antibodies: current status and outlook for the future, *Alzheimer's Research & Therapy*, 2013, **5,** 56.

[2] SpotLight Proteomics: uncovering the hidden blood proteome improves diagnostic power of proteomics: S L. Lundström, B Zhang, D Rutishauser, D.Aarsland & **R. A. Zubarev**. Nature Scientific Reports | 7:41929 | DOI: 10.1038/srep41929

[3] Fornelli L, Damoc E, Thomas P M., Kelleher N L., Aizikov K, Denisov E, **Makarov A, Tsybin Y. O.** Top-down analysis of monoclonal antibody IgG1 by electron transfer dissociation Orbitrap FTMS. Mol Cell Proteomics (2012) 11:1758-1767.

[4] Fornelli L, Ayoub D, Aizikov K, Liu, Damoc E, Pevzner P, **Makarov A,** Beck A, **Tsybin Y**. Top-down analysis of immunoglobulin G isotypes 1 and 2 with electron transfer dissociation on a high-field Orbitrap mass spectrometer. J Proteomics (2017) 159, 67–76.

[5] Fornelli L, Ayoub D, Aizikov K, Beck A, **Tsybin Y O**. Middle-down analysis of monoclonal antibodies with electron transfer dissociation Orbitrap FTMS. Analytical Chemistry (2014) 86, 3005-3012.

The targeted breakthrough of the TopSpec is its unique ability to sequence whole intact antibodies. This breakthrough could substantially reduce the development costs of new drugs and dramatically reduce the time to market.

The specific TopSpec objectives are:

- **Sequence** - **The biggest problem in top-down MS/MS: sequencing of large proteins,** will be solved by implementing novel gas-phase radical reactions in the ground-breaking MS/MS device, the Omnitrap. An ion mobility (IM) device will be attached to it, providing hardware deconvolution of overlapping isotopic clusters that present a daunting problem for deconvolution algorithms. The ions will then be detected by an ultra-high resolution Orbitrap mass analyser with extended functionality.
- **Analyse** - **To solve the next-biggest problem in top-down MS/MS: implementing novel deconvolution procedure to attribute isotopic peaks to individual molecules or fragments.** The custom-designed FT Booster will offer the most modern advances in signal acquisition and real-time data processing, will increase the MS/MS spectra quality and radically simplify them via our breakthrough deconvolution algorithm.
- **Optimize** - **To optimize the front-end, online separation of large proteins,** by replacing the conventional (and poorly suitable for large proteins) high-pressure reversed-phase liquid chromatography (LC) with a revolutionary novel low-pressure separation device called the *pI-Trap*.
- **Combine** - **To combine in a seamless way** the above components into a TopSpec instrumentation platform by designing sophisticated software to control the whole platform as a single apparatus. Novel software for data acquisition, processing and analysis, including *de novo* protein sequencing, will be created.
- **Utilize** - **This will then allow us to create novel top-down strategies** that will fully utilize the analytical power of TopSpec to sequence large proteins, and to implement these strategies for solving disease-related problems.

1.3 Interdisciplinarity and non-incrementality of the research proposed

There is no way to personalized medicine without solving the major challenge - the individual character of a person's immune system. Only by deciphering the Ab repertoire can we understand how an individual organism reacts to disease and drugs. The task is immense, yet the previous experience in proteomics is that, if one starts with most abundant species, the early success feeds fast and robust progress. Sequencing 100 most abundant Abs in 500 AD patients vs 500 controls would revolutionary improve our understanding of that severe disease.

Current state of the art

LC-MS/MS is a key technique in the analysis of individual proteins as well as whole proteomes (proteomics - large-scale protein analysis). Current state-of-the-art Ab analysis uses a *bottom-up* approach, which is based on MS/MS sequencing of the peptides obtained after S-S bond reduction and enzymatic digestion of the Ab. However, an incomplete population of peptides is usually detected, with losses of molecules that are either modified, or outside the range of mass or hydrophobicity. Moreover, even if, hypothetically, 100% sequence is recovered, peptide alignment in the complementarity determining regions (CDRs) remains a challenge. Since these highly variable regions are complementary binders to the antigen of interest, their sequencing is crucial. Implementation of multiple enzymes to obtain partially overlapping peptides gives no guarantee of success. Moreover, Ab digestion into peptides destroys information on the connectivity of heavy and light chains. The solution is to keep proteins intact before MS analysis, and perform sequencing in the gas phase (this is known as *top-down MS/MS approach*).

Complete sequencing of intact proteins of >20 kDa (about 200 AA) still represents an insurmountable challenge to MS, mainly because of the restrictions imposed by MS/MS techniques as well as inability to decipher all molecular information from mass spectra exhibiting massively overlapping isotopic distributions. Traditional collision-activated dissociation (CAD), higher-energy collisional dissociation (HCD) and electron transfer dissociation (ETD) employed in Orbitrap and time-of-flight (TOF) mass spectrometers do not produce cleavage of all inter-residue bonds. Even UV photons employed in top-down MS/MS of Abs cleave only 30-40% inter-residue bonds at best, which is far from sufficient. Some attempts to cleave an Ab into few larger (20-50 kDa) parts in a *middle-down* approach has shown promise, but is still a long way from success in terms of full sequence coverage. The simple fact is that **more energetic and diverse MS/MS fragmentation techniques are needed!** The most promising are the *non-ergodic reactions*, where bond breakage occurs faster than energy redistribution. An example of such a technique is electron-capture dissociation (ECD)[6], in which multiply charged protein polycations capture low-energy electrons. However, ECD releases only 4-7 eV of recombination energy, which is not enough.

Another great challenge is data analysis. The most useful m/z range in FT mass spectra is from 200 to 2000, while the masses of Ab fragments are from 200 Da to 150,000 Da, and the charges range from 1+ to ≈75+. Because of the stable isotopes, such as ^{13}C, ^{15}N, etc., every molecule has an isotopic distribution, with the width varying from

[6] (ECD - Zubarev, R. A.; Kelleher, N. L.; McLafferty, F. W. *Electron Capture Dissociation of Multiply Charged Protein Cations. A Non-ergodic Process, J. Am. Chem. Soc*. 1998, *120*, 3265)

2 Da to ≈25 Da. Every inter-residue link can be cleaved in 3 different ways, producing up to six fragments. Thus the MS/MS spectrum is literally stuffed with hundreds of overlapping fragments ions, which makes it impossible to identify all, and even most, of them.

Beyond state-of-the-art: **TopSpec - The most sophisticated platform for top-down analysis of intact proteins.**

To address these challenges, we envision an unique instrumental platform that combines novel fragmentation methods in MS/MS with ion mobility and a truly innovative ion deconvolution approach, as well as the best achievements in front-end separation merged with ultrahigh-resolution MS (Fig. 1). This will allow us to achieve the elusive 100% sequence coverage in top-down analysis of human Abs.

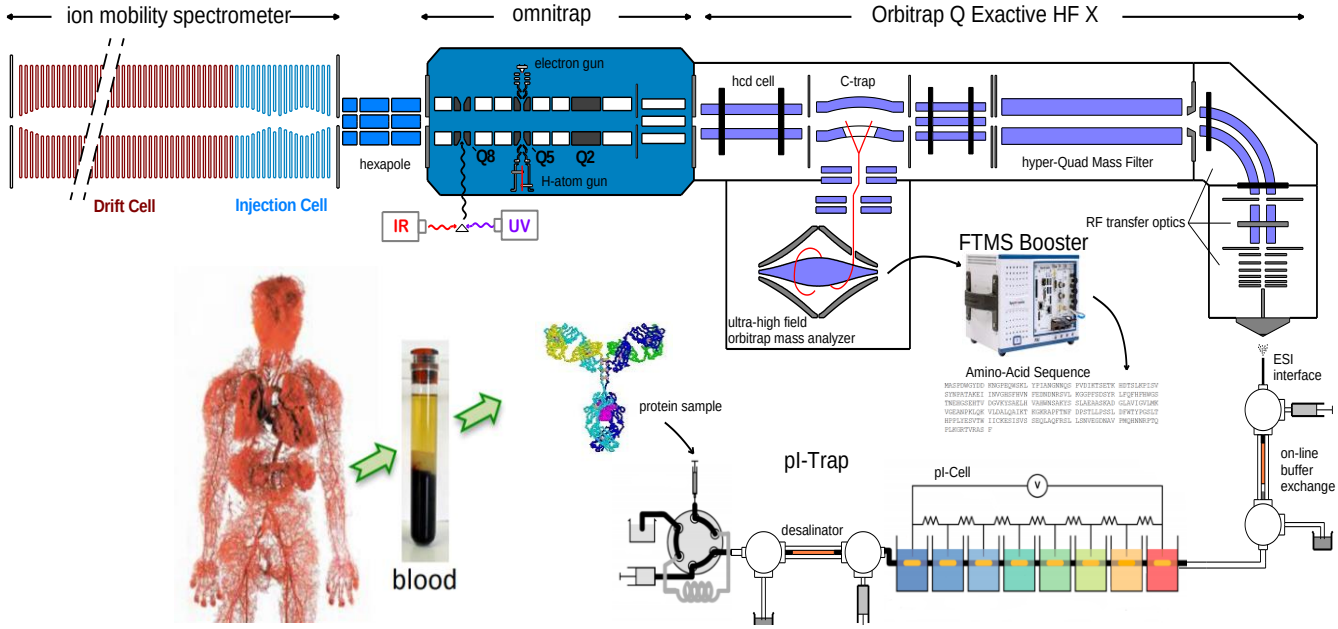


Fig. 1. TopSpec design*. pI-Trap online pI fractionator (**BM**), incl. desalinator, capillary isoelectric focusing, online buffer exchanger and ESI interface; modified Q-Exactive HF X ultra-high-resolution FT mass spectrometer (**TF**); FTMS Booster – high-performance data acquisition and real-time big data processing system (**SPS**); Omnitrap – all-inclusive MS/MS device for protein sequencing (**FASM, KI**); ion mobility set-up (**FASM**).*

1. The antibodies will be enriched from human blood (**KI, IP**), and intact Ab molecules will be loaded into the pI-Trap, which is a new innovative micropreparative device that has been developed by Biomotif (**BM**). Upon desalting in an online desalinator, pI-Trap will separate Abs by their isoelectric point (pI) in solution. After on-line buffer exchange, they are ionized and transferred into the gas phase by electrospray ionization (ESI). The desalination step is critical for obtaining good pI fractionation, as well as for obtaining high abundance of molecular ions to enable MS/MS sequencing.

2. The Ab ions then enter the Orbitrap MS, where they are selected by m/z in an enhanced quadrupole filter of an improved Q Exactive HF Orbitrap FT MS (**TF**) and then sent onwards to the Omnitrap via the C-trap.

3. The Omnitrap (**FASM, KI**) is a novel unique device, unprecedented in its flexibility and capabilities. Being attached to the back end of the Orbitrap MS, it employs a hexapole ion guide followed by nine (!) serially coupled linear ion traps, with a full arsenal of ion manipulation abilities, such as *m/z* isolation, radial and translational ion motion excitation, and transfer between the traps in any order. The Omnitrap will be equipped with an electron gun, hydrogen atom source, as well as UV laser, making it capable of performing all possible MS/MS fragmentation techniques, such as electron bombardment with attachment (ECD, ≈0 eV), excitation (1-10 eV) and ionization (10-1000 eV energy range), hot hydrogen atom bombardment, plasma bombardment, UV photo dissociation, collisional dissociation both in the low- (CID) and higher-energy (HCD) ranges. Importantly, these MS/MS (MS2), MS/MS/MS (MS3), and MS/MS/MS/MS (MS4) techniques as well as bombardment by hydrogen atoms and radicals can be combined in any order, starting from HCD MS/MS that is already implemented in the Orbitrap (Fig. 2). The Omnitrap will be able to perform multi-notch selection of several charge states of protein ions simultaneously, which will progressively improve the analysis sensitivity, as large molecules appear in ESI in many charge states simultaneously. After ion excitation and fragmentation by novel MS/MS techniques, the remaining precursor ions will be selectively removed to reduce the dynamic range problem and facilitate the detection of low-abundant fragments. The Omnitrap will also be capable of accumulating the MS/MS products from several MS/MS events for subsequent simultaneous detection with enhanced signal/noise. The instrument control software (**FASM, TF**) will allow the Omnitrap to operate seamlessly with the Orbitrap mass spectrometer.

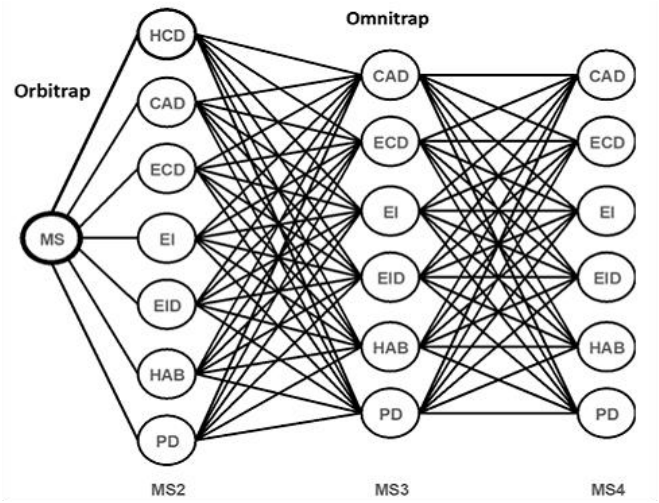


***Fig.2**. The Omnitrap MS/MS and electron-, radical-reaction capabilities: HCD - higher-energy collisional dissociation; CAD - (lower-energy) collisional dissociation; ECD - electron capture dissociation; EI - electron ionization (without dissociation); EID - electron ionization dissociation (family); HAB - hydrogen atom bombardment (family; may result in - alternatively or concomitantly - attachment, exchange and dissociation); PD - photo-dissociation*

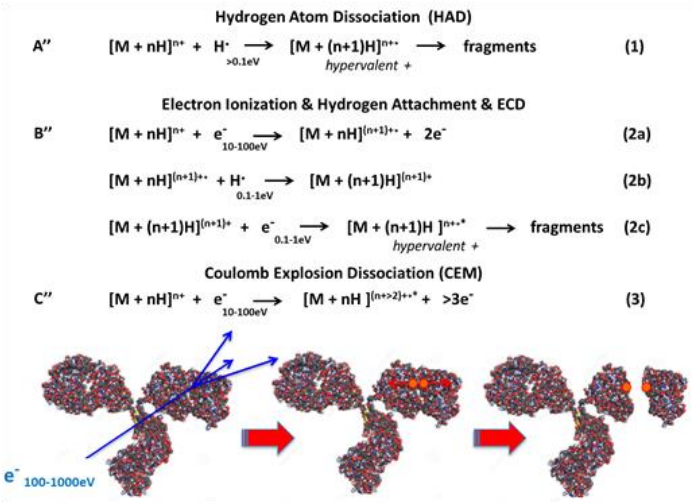


***Fig. 3.** Novel reactions that are planned to be implemented in the Omnitrap - Hydrogen atom bombardment (HAB) family: A) Hydrogen atom dissociation, HAD. B) Electron ionization followed by hydrogen attachment followed by ECD; and electron ionization family: C) Coulomb explosion dissociation, CED.*

Novel ion fragmentation techniques implemented in the Omnitrap (**KI, FASM**) will be much more energetic than ECD, and they will also form hypervalent hydrogen-abundant protein radicals that will non-ergodically fragment to produce a variety of sequence-specific fragment ions. The aim is also to obtain such hydrogen-abundant radicals in the same or higher charge state as the precursor ions, in order to increase the sensitivity.

4. A very important fragmentation reaction involves the attachment of hydrogen atoms (H•) to ions. Preliminary results indicated that such a process is only efficient for hyper-thermal (≥0.1 eV) H•. Thus we will build a source of unprecedentedly fast, ≥1 eV H• (**FASM, KI**) (Fig. 3A). Another group of reactions will use charge state increase of ions by >10 eV electrons, forming hydrogen-deficient radicals, which will be converted by addition of hot H• to protonated molecules. ECD of such molecules will produce hypervalent species of *the same charge state as precursor ions* with subsequent nonergodic fragmentation (Fig. 3B). With >100 eV electrons, multiple charge state increase will result in a Coulomb explosion (which has so far only been observed on small molecules), resulting in immediate dissociation (Fig. 3C) with the *homogeneous* fragmentation of all inter-residue bonds. IR and UV lasers will provide vibrational and electronic excitations, respectively.

5. To facilitate the analysis of the multitude of fragment ions in different charge states overlapping on the m/z scale, ion mobility (IM) gas-phase fractionation will be implemented. IM uses the fact that the low-energy collision cross section of ions is higher for higher masses and charge states. Thus fragment ions from the Omnitrap will be "dragged" through collision gas in the IM device and fractionated by their collisional cross sections into 3 fractions (Fig. 4-left). Each fraction will be sent to the Orbitrap for detection and analysed individually.

6. To accommodate the extreme information flow, the Orbitrap ion detector will be equipped with a novel version of the FTMS Booster (**SPS, TNTU**) for increased sensitivity, resolution and mass accuracy. Critically, low-abundant signals will be kept. FTMS Booster works in parallel with the conventional ion detection system of the Orbitrap, ensuring system robustness. The device uniquely will store signal transients, allowing one to implement the novel deconvolution strategy (Fig. 4-right) based on the fact that the signal transient decays due to high-energy collisions with residual gas, and the decay rates for the individual ions of an isotopic cluster are similar, and thus can be clustered together using their decay constants. The latter are measured in post-processing of the transients acquired by FT Booster (the standard Orbitrap software does not allow to store and post-process transients).

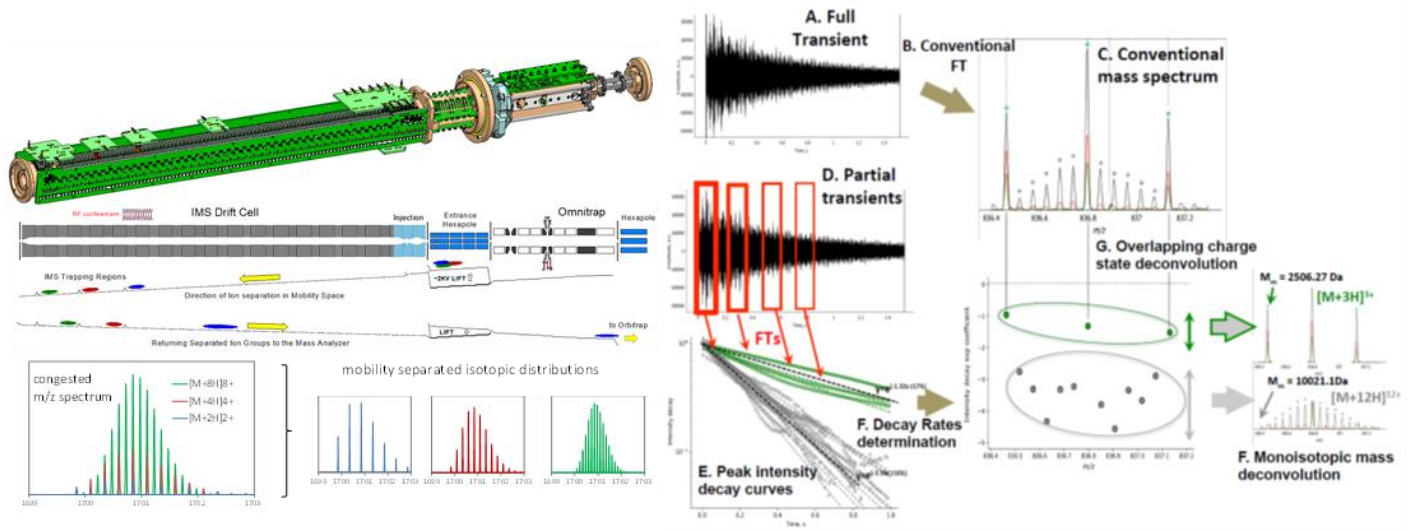


Fig. 4*. Novel strategies for top-down MS/MS signal deconvolution: Left – ion mobility (IM) device separating fragment ions by collisional cross-sections into 3 fractions, which are re-sent to Orbitrap in separate packets; Right – novel post-acquisition deconvolution of collisional cross sections in high-energy collisions with residual gas in the Orbitrap: A: full extended-length signal transient; B and C: it is converted by FT to conventional overlapping mass spectrum; D, E, F: partial transients provide signal decay curves for individual m/z peaks; G: decay constants of the isotopic peaks of the same molecule are clustered together; F: conventional deconvolution of isotopic distributions provide accurate monoisotopic masses of the molecules.*

Non-incrementality of the procedure and approach to be developed and implemented

1. Novel reactions will transform top-down mass spectrometry. Novel fragmentation techniques and advanced data processing tools will allow ***unique analysis capabilities*** using Top-down MS technologies.

2. Using TopSpec will dramatically increase knowledge about human antibody repertoire and the immune system. To enable full understanding of how immune system reacts to a particular disease challenge

3. Using TopSpec will shorten the path to precision, personalized medicine, possibly even by years, by providing individual's blood antibody profile. Identification of a specific Ab sequence raised by an individual against a challenge (invasion or disease) will immediately provide a clear way to individual therapeutic antibodies.

How we will achieve a Targeted Breakthrough

Breakthrough 1 *– Omnitrap – the most powerful MS/MS set-up in history of mass spectrometry.*

Breakthrough 2 *– IM and transient-centric decay constant deconvolution for in-depth top-down analysis.*

Breakthrough 3 *– Novel front-end separation approach for top-down protein analysis.*

Research disciplines supporting the targeted breakthrough of the project: The project unites physicists, chemists, biologists, engineers, software developers, bioinformaticians, communicators and managers, at top academic institutions, SMEs and a large vendor company. Many young scientists are also working in the project.

Added value of interdisciplinarity: TopSpec will be much more than the sum of individual parts, capitalizing on the latest developments in such diverse areas as personalized medicine, biochemistry, liquid separation, MS and MS/MS instrumentation, gas-phase fragmentation, data processing, mathematics and bioinformatics.

1.4 High risk, plausibility and flexibility of the research approach

Antibody isolation: Abs from human blood will be enriched to >50% by Melon Gel that binds non-Ab proteins. Tandem Melon Gel isolation increases the Ab content to >80%; triple isolation – to >95%.

The pI-Trap system: human blood has high salt content (≈0.9% NaCl), which is detrimental for both isoelectric focusing and MS analysis. Thus, samples will be desalted in the pI-Trap desalinator using a dialysis Nafion membrane tube. Then sample molecules will be fractionated by pI inside the pI-cell, a multiple junction capillary divided into eight equal segments, interconnected by Nafion membrane tubes. The buffer solution in each segment has its own pH, which is also present inside the membrane tubes, and thus a pH gradient is established. The voltage gradient across the pI-cell is created by the inserted platinum electrodes connected to a HV power supply. After 10-60 min of focusing, the proteins are mobilized by buffer flow actuated by a syringe pump. Upon elution, the buffer is replaced by ESI solvent in an online buffer exchange device, to easily desorb/ionize proteins by ESI.

The Omnitrap-Q Exactive combination: The Q Exactive HF-X Orbitrap mass spectrometer (**TF**) will be equipped with an expanded mass range up to Abs in the native state. New hardware and software (**FASM**) will and transfer

the ions without losses between the two instruments. The Omnitrap is driven by rectangular RF waveforms, to ensure full digital control of potentials. Sweep-type waveforms will be used for ion isolation with single or multiple notches in the segment Q2. Q2 will also perform slow heating collision-activated dissociation, CAD, accomplished by pulsing heavy gases (Ar or Xe) for optimal collisional activation. Q5 will support ion-electron reactions enabled by a 0-1000 eV electron source. Ion-radical reactions will use a novel hydrogen atom source with an unprecedented range of kinetic energies (0.05 eV to ≥1.0 eV. Q8 provides the photo-excitation by IR and UV lasers; it could also accumulate huge fragment ion populations for their detection in the Orbitrap. All activation-dissociation tools can be applied in any combination (Fig. 2). The Omnitrap will be equipped with three independent pulse valves for a truly diverse set of experiments. The reactions shown in Fig. 3 will be developed.

The Omnitrap-IM combination: The spectral congestion in MS/MS spectra will be addressed by a new method that utilizes a high performance ion mobility drift cell combined with the Omnitrap platform (**FASM**). Fragment ions, which in a typical top-down experiment comprise >1000 distinct isotopic distributions simultaneously confined in limited m/z range, will be injected into a pressurized drift cell. Fragment ions, being dragged through gas by electric field, will separate according to mobility, which depends on their mass, shape and charge, and will be divided into 3 fractions, which will be stored in 3 separate back-end trapping region. New developments in electronics will allow reversing the DC gradient across the drift cell and re-injecting each discrete group separately into the Orbitrap for m/z analysis in cycles of <100 ms. This is expected to greatly reduce spectral density.

The FTMS Booster for advanced ion signal detection and processing (SPS, TNTU): this unique advanced signal acquisition and real-time analysis system **(SPS)** will be further enhanced for higher speed and accuracy. It will record and store the time-domain signals (transients) all the time when ions are trapped in the Orbitrap, uniquely enabling acquisition of extended transients (1-3 s duration), which is crucial for implementation of decay constants approach for mass spectra deconvolution. The arsenal of off-line re-analysis techniques includes absorption mode Fourier transform, linear regression, least-squares fitting, filter diagonalization, phased spectrum deconvolution, and compressed sensing, which will result in unprecedented sensitivity, resolution and mass accuracy.

Software development: Integrating controlling software (**KI** and **FASM**), raw data processing software (**SPS)**. Novel top-down MS data analysis software (the existing software is grossly inadequate) - **KI**, **IP**, **SPS** and T**NTU.**

Sex/gender aspect: There is an ≈40:60 female: male ratio amongst staff in 8 partners. When recruiting, we will apply all national laws and the European Charter for Researchers to ensure equal chances for effective career development for all. The newly appointed project manager (senior scientist and antibody specialist Susanna Lundström, KI) is female. Prospectively, we aim to further improve the gender balance in other prominent key roles in the consortium.

Biological samples: The risk associated with the translation of results into real biological samples is rather low, as we have access to antibodies related to diseases where the whole antibody repertoire is dominated by a small subset of antibodies and even by a single clone.

2. Impact

2.1 Impact on technology and society

The transformational opportunity: If successful, TopSpec will greatly expand our knowledge of the human immune system, which may have a dramatic impact on the field of personalized, precision medicine. TopSpec may facilitate the development of new diagnostics and treatments for infectious diseases including global diseases and the problem of treatment resistance, ageing related diseases (e.g., AD) and other big killer diseases. Another significant impact will be in the field of MS instrument design.

Specific impacts:

- Increase in the speed of diagnosis and in the speed of drug development
- Increase knowledge on an individual´s antibody response to disease
- Contribute to the growth and expansion of 4 European SMEs
- Expand scientific research around proteomics
- Create new business opportunities within and outside the project

Sustainability and equity of the European Health system: A major challenge in all health systems is the cost of drugs and targeted therapies. Reducing time taken to develop novel therapies, will reduce costs to the health system.

2.2 Impact on future leadership

Innovation across the European Research Area- Creation of new research communities: The TopSpec will stimulate creation of new scientific fields in the areas of a) fundamental gas-phase protein chemistry; b) protein mass

spectrometry and proteomics; c) immunology; d) therapeutic areas. This will help EU researchers and companies to take the lead in the respective fields, and lead to the development of novel targeted therapeutics.

Industrial leadership: Innovation and market opportunities for the EU biotech industry: Of the eight partners, four are European SME partners and one is large industry partner. For each SME, the TopSpec project will result in novel technologies that will give the companies new commercial opportunities, expansion and market leadership.

2.3 Measures for achieving impact

a) Dissemination and exploitation of results

Draft plan: The results and data will be presented at industrial and academic conferences, user meetings, immunology, proteomics and MS conferences. Early adopters have been contacted: the Swedish National Centre for Biological Mass Spectrometry (Bio-MS); the Proteomics centre of the Copenhagen University (Prof. Michael Nielsen); Proteomics centre, University of Southern Denmark, Odense (Prof. Frank Kjeldsen); Protein analysis laboratory of Novo Nordisk AS, Copenhagen (Dr. Kim Haselmann); Protein analysis laboratory of Amgen (Dr. Pavel Bondarenko); Proteomics laboratory of Harvard University, FAS Division of Science Core Facility (Dr. Bogdan Budnik); Proteomics laboratory of Stanford University (Dr. Chris Adams); Top-down protein analysis laboratory of Innsbruck University, Austria (Dr. Kathrin Breuker); NIH-supported glycoproteomics laboratory at Boston University (Prof. Catherine Costello).

Measures for protection of intellectual property: A detailed description of the IP background will be found in the consortium agreement (CA). Management of IP issues will follow the DESCA 2020 model CA tailored to the specific interests of all consortium partners.

Expected Result	**Target Industry**	**Use within the project**	**Use outside the project**
Novel MS/MS platform	MS instrumentation	Adopted by TF	Adopted by other MS manufacturers
Top-down Ab sequencing assay	Biotechnology, Clinical diagnostics	Proof of principle, biomarkers of AD and bacterial infection	Quality control in mAb production, biosimilars and biobetters, clinical diagnostics
Library of Ab repertoire as immune system response to challenge	Immunology, Bioinformatics	Proof of principle	Large EU projects to collect Ab sequence libraries for specific diseases
Top-down data analysis software	Analytical, biotechnology, pharmaceutical	Proof of principle	Open source and commercial versions for industrial and academic analytical scientists
Novel data acquisition and real-time data processing system	MS instrumentation	Adopted by Spectroswiss	Adopted by other MS manufacturers, including TF

Open research Data Management and strategy for knowledge management and protection:

TopSpec	**Description**
Data and routines	Scientific reports, test results and data. Data storage and collection routines will be agreed and documented at the start of the project, to facilitate exchange of data
Internal routines	Each partner will be responsible for maintaining their own datasets, which will be made accessible to all partners. Data will be made publicly available (subject to IP and publication factors).
Exploitation of data	Ensure long-term preservation of data. Ownership and exploitation of data – see CA. Exploitation strategy will be made for each dataset by the IP-owning partner (s) or by the whole consortium.

b) Communication activities

In addition to the consortium website, the results and data will be regularly discussed per email, conference calls and meetings (WP 9). Seven biannual consortium meetings are planned; will be held at partner sites in conjunction with key milestone achievements, so that young scientists are actively involved in on-site presentations.

Public communication measures to facilitate exploitation:

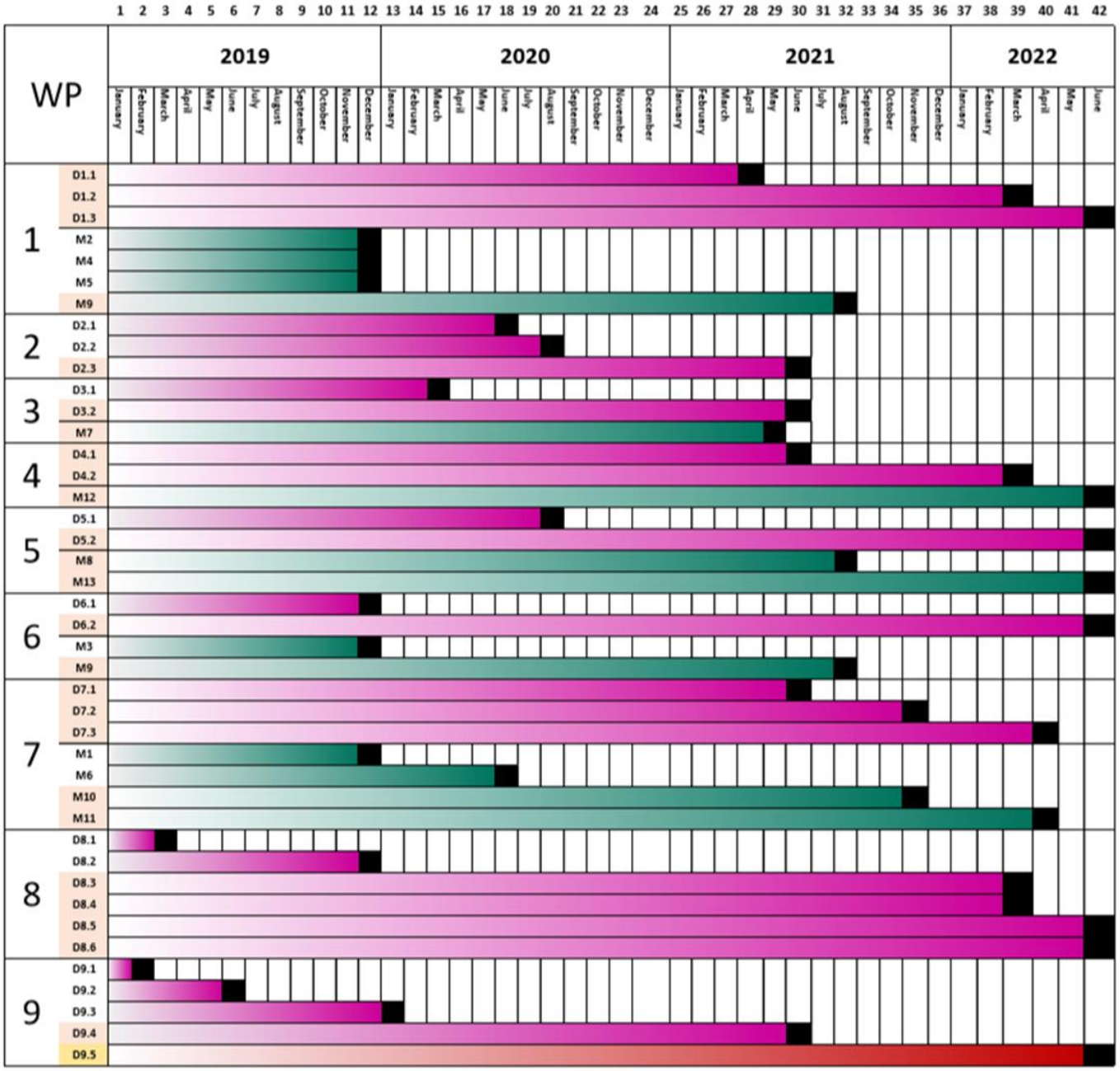
Target groups	**Communication/Dissemination action**
Website	Publicly accessible website with a closed partner access.
Proteomics research community	Collaborations, scientific reports. Open access publication in relevant journals: Analytical Chemistry, J American Society for Mass spectrometry, JACS, Molecular & Cellular Proteomics, J of Proteomics, Nature Meth and Nature Biotechnology.
Young scientists	Young scientists will be encouraged and promoted. Exchange of young researchers will be organised. Summer schools and workshops.
Health care providers	Focused meetings to bring technology developers and end users together. Video demonstrations accessible through YouTube and through partner websites
Diagnostics and pharma industry	Ongoing collaboration with Amgen, Astra-Zeneca, Bayer, Sanofi, other EU pharma companies. International conferences: Bio, EuPA and HUPO meetings, IMS and ASMS conferences, BIT congresses, PittCon, Analytica. National meetings, CASSS-meetings.
General public and broader audience	Information through website, social platforms (Linkedin, Twitter) mass media (newspapers, TV, radio) popular science journals, press releases. Open exhibitions.

3. Implementation

3.1 Work plan and intermediate targets

Overall structure of the work plan: There are 7 technical WPs for building and testing of the proposed platform. As shown in Figure 1, the TopSpec design includes the development of several different platforms that combined will improve intact (oligo- and polyclonal) Ab characterization. For protein clean-up, fractionation and ionization WP6 (Development of pI Trap-ESI combination) is needed. The modified pI trap will be in-line connected to a mass spectrometer that combines the omnitrap (WP 1-4) and the modified Oribtrap mass spectrometer (WP6), which combined will be designed to improve and optimize the anybody characterization. WP7 is then dealing with improvements in Top down-MS-data interpretation (signal detection and data processing) of the analyzed proteins. WP 8 focuses on dissemination and exploitation activities, and WP9 describes its management and administration.

3.1.1 Timing of the work packages (Gantt chart). Due to the COVID-19 pandemic, we request a 6 months extension of the project. For this reason, several of the deliverables and milestones have been changed to later due dates as highlighted in the Gantt chart in the WP column. Furthermore, an additional scientific review meeting has been incorporated (Deliverable, D9.4, month 30) and the final reporting period (D9.5) has been changed from December 2021 to June 2022. **WP1:** Omnitrap development and testing **WP2:** Implementation of CAD, ECD, HECD, UV, IR PD, and EID MS/MS techniques in Omnitrap, **WP3:** Development and application of H-atom bombardment (HAB) MS/MS techniques, **WP4:** Development and application of Coulomb explosion MS/MS technique, **WP5:** Development of pI-Trap-ESI combination, **WP6:** Modification of the Orbitrap mass spectrometer, **WP7:** Signal detection and data processing **WP8:** Dissemination, Communication & Exploitation, **WP9:** Project Management and Administration. **D:** deliverables, **M:** Milestones.



3.2 Management structure, milestones and procedures

The Project Coordinator, prof. Roman Zubarev, will chair project meetings and the steering group meetings, direct the progress of the project, and support the WP leaders. A Project office will perform the day to day management, communication between partners, website, meetings etc. The steering group has one representative from each partner. Decisions are made by a majority. Each WP leader is responsible for the WP and tasks; is responsible for collection of results and data, and compilation of reports. All Partners: fulfill their tasks on time and within budget, and provide reports to WP leaders. All contribute to dissemination.
We plan that the Dissemination and Exploitation Plan will be developed by the end of the first Reporting Period (RP1, month 12) and that it will be updated for the subsequent Reporting Period at 36 months. Management procedures will follow qualified majority. Consortium meetings will take place at least twice a year. Video conferences will take place every month. Ad hoc video conferences should be possible.

3.3 Relevance of expertise in the consortium

Three members are EU universities (**KI, IP, TNTU**). Young scientists are both PIs and team scientists. The expertise from commercial partners, from SMEs to large commercial entities, includes engineering (electrical, mechanical, chemical), software development, chemists, and application scientists. Software and hardware developers, analytical scientists and software developers. Institute Pasteur (IP) is the historical cradle of the antibody research, and having them part of our consortium ensures that the experts are present. The fact that they are also experts in top-down sequencing of proteins is a perfect match of different expertise we need.

Table 3.4 b: 'Other direct cost' items

1/KI	Cost (€)	Justification
Travel	10 000	Partner meetings, Conference visits, scientific exploitation actions. An average 1000€ per meeting.
Equipment	30 000	UV Laser (€20,000), IR Laser (€10,000)
Other Goods and Services	64 000	Consumables for MS (solvents & buffers; chemicals, columns, tips, tubes, vials), 25 000 Euro, gases and other consumable for lasers, 4 000, consumables for sample prep (solvents & buffers; chemicals,, Melon Gel) 15 000, software licences (data processing, analysis) 15 000, Audit costs, 5 000
Total	104 000	
2/FASM	Cost (€)	Justification
Travel	6 000	Biannual Partner meetings, Travel to KI, for conference attendance, and business exploitation actions. An average price of 1000 EUR per meeting has been calculated.
Equipment	20 000	Test and Measurement equipment, Omnitrap and lab PCs. 4x HV psus (6000), 2x oscilloscope probes (3000), 4x Omni&Lab PCs (6000), 1x spectrum analyzer (3000), 1x programmable electronic loads unit (2000)
Other Goods and Services	306 000	Raw materials, vacuum kits, mechanical and electronics for 2x H-atom gun construction (80k€) Raw materials, vacuum kits, mechanical and electronics for 2x Omnitraps (140k) Raw materials, vacuum kits, mechanical and electronics for one IMS (60k€). Replacement mechanical & electronics parts (20k€) Audit certificate (6k€)
Total	332 000	
3/ TF	Cost (€)	Justification
Travel	5 000	Partner meetings. Average 1000 EUR per meeting.
Equipment	350 000	Q Exactive HF -X at cost price.
Other Goods and Services	25 000	Gases for the lasers and MS, solvents and buffers, plastic ware, glassware, standard compounds (20 000), Audit certificate (5000)
Total	380 000	
4/SPS	Cost (€)	Justification
Travel	10 000	Partner meetings, conferences, business exploitation actions (1000 EUR per meeting).
Equipment	80 000	Parts (e.g., electronics modules) for development and production of 2 FTMS Boosters for top-down MS.
Other Goods and Services	18 000	Cost split: 13 000 Euro: consumables (cables and contacts, data storage devices), 5 000 Euro: audit certificate.
Total	108 000	
5/BM	Cost (€)	Justification

Travel	10 000	Biannual Partner meetings, Congresses/BD actions.
Equipment	112 000	Installation of 2 pI-Traps. 2 x autosampler-fraction collectors (50.000 Euro) 2 set of power supplies and voltage dividers (16.000 Euro), 2 x PC system with Special software modules (12.000 Euro) , Valves and injectors including special tubing (6.000 Euro), syringe low flow rate pumps (3.000 Euro) Metal housing, frames, cabling and contacts (10.000 Euro) , pI Cells with cell holder and platinum electrodes (15.000 Euro)
Other Goods and Services	30 000	Solvents, buffers, pure chemicals, ultrapure gases for MS and MS/MS, standards.
Total	152 000	
6/TNTU	Cost (€)	Justification
Travel	10 000	Partner meetings and Conferences
Equipment	6 000	Test and Measurement equipment
Other Goods and Services	24 000	Consumables (e.g. cables, storage devices, standard materials) 6 000 Euro, software licenses (data processing, analysis, visualization, modelling etc.) 18 000 Euro
Total	40 000	
7/IP	Cost (€)	Justification
Travel	6 000	Partner meetings and Conferences
Equipment	30 000	UV Laser (20 000), IR Laser (10 000)
Other Goods and Services	32 000	Consumables for MS (solvents & buffers; chemicals, Melon Gel, columns, tips) 20000 Euro, gases and other small consumable for lasers (2000), software licences (data processing, analysis) (10000)
Total	68 000	
8/MS	Cost (€)	Justification
Travel	6 000	Partner meetings and Conferences (€1000 per meeting)
Other Goods and Services	10 000	Consumables, mechanical & electronic parts
Total	16 000	

Section 4: Members of the consortium

List of participants

Participant Nº	**Lead Name**	**Organisation/Country**	**Short name**
1	Roman A Zubarev	Karolinska Institutet/Sweden	KI
2	Dimitris Papanastasiou	Fasmatech Science and Technology SA/Greece	FASM
3	Alexander Makarov	Thermo Fisher Scientific, Bremen/Germany	TF
4	Yury Tsybin	Spectroswiss Sàrl/Switzerland	SPS
5	Thorleif Lavold	BioMotif AB /Sweden	BM
6	David Kilgour	Nottingham Trent University, UK	TNTU
7	Julia Chamot-Rooke	Institut Pasteur/France	IP
8	Jan Commandeur	MS Vision/Netherlands	MS

4.1 Participants

4.1.1 Participant 1 (Co-ordinator)

Participant number	**1**
Organization full name	Karolinska Institutet
Organization Short name	KI
Website	www.ki.se

Description of the institution

Karolinska Institutet, Stockholm/ Sweden (www.ki.se) is one of the world´s leading medical universities. KI accounts for over 40 percent of the medical academic research conducted in Sweden and offers the country's broadest range of education in medicine and health sciences. Since 1901, the Nobel Assembly at Karolinska Institutet has selected the Nobel laureates in Physiology or Medicine. KI has several campuses in different areas of Stockholm. Campus Solna has 1,709 FTE students, receives 1,634 million SEK for research annually, and employs 1,990 FTE employees. Campus Huddinge and Karolinska University Hospital, Huddinge have 3,074 FTE students, receive 904 million SEK for research and employ 1,081 FTE employees, while Karolinska University Hospital, Solna has 756 FTE students, obtains 1,280 million SEK for research and has 1,149 FTE employees.

The Department of Medical biochemistry and Biophysics (MBB) is one of the largest of KIs 20 departments, with 18 full professors, and is one of the academically most successful (three Nobel prize laureates have worked here). MBB has the oldest mass spectrometry facility in biomedical sciences in Europe and the second oldest in the world, which opened its doors for service in 1947. Today the mass spectrometry facility has 9 operational mass spectrometers, including six high-resolution Orbitrap mass analyzers. The research activities of the laboratory have a broad scientific scope, including method development for proteomics.

Key persons carrying out the research

Roman A Zubarev (RAZ) (male) (PhD) is a full professor, chair and director of the Chemistry I division in the department of medicinal biochemistry and biophysics at the Karolinska Institutet/ Sweden.

Prof Roman A. Zubarev (RAZ) studied applied physics at Moscow Engineering Physics Institute where he obtained his Masters´ degree. He earned his PhD from the Uppsala University (Sweden) in the field of Ion Physics and did his postdoctoral training in Fred McLafferty's lab at Cornell University (United States). He became associate professor of biological mass spectrometry in Odense (1998), before moving back to Uppsala in 2002 as a professor of proteomics. In 2008, he accepted KI's offer and moved to Stockholm. He has a broad research interest. He has discovered Electron capture dissociation (ECD) (1997) and other ion-electron reactions (1998-2008) for the analysis of polypeptides by mass spectrometry; formulated the Isotopic Resonance hypothesis of the origin of terrestrial life (2008); experimentally verified the Isotopic Resonance phenomenon (2011-2014), and formulated the Isoaspartate hypothesis of the origin of Alzheimer's disease (2011), as well as developed the "pI=7.4" method of biomarker discovery. His current research includes developing mass spectrometry based tools for studying disease mechanisms and developing novel proteomics tools.

He has authored more than 260 papers cited more than 5000 times (without self-citation); h-index is 50 (Web of Science) or 56 (Google Scholar) and owns over ten patents. He has supervised as a main supervisor 9 PhD students, of which two are currently professors, and two – staff scientists (Harvard and Stanford). He is recipient of the Distinguished New Technologies Award at RECOMB (Venice 2006), the Curt Brunnée Award from the International Mass Spectrometry Society "for outstanding contribution to the development of mass spectrometry instrumentation"(Prague 2006), the Klaus Biemann Medal for "a significant achievement in basic or applied

mass spectrometry made by an individual early in his or her career" from the American Society of Mass Spectrometry (Indianapolis (USA) 2007) and the Gold medal from the Russian Mass Spectrometry Society (2013). He has received "Outstanding" ranking in ERA2010 evaluation, among the top 14% of all KI professors (2011).

Assigned project manager and senior scientist Susanna L Lundström earned her PhD at Karolinska Institutet 2008 in the field of structural characterization of biomolecules using mass spectrometry and NMR techniques. She has published over 20 peer review articles as well as reviews and a book chapter and hold a current H-index of 11 (from web of knowledge). Over the last seven years the main focus of her research has been on mono- oligo- and polyclonal antibody sequencing and characterization.

An experienced EU-project financial manager from KI's central Grants Management Office will be appointed.

Relevant publications, products or services

1. **Zubarev, R. A.;** Kelleher, N. L.; McLafferty, F. W. *Electron Capture Dissociation of Multiply Charged Protein Cations. A Non-ergodic Process, J. Am. Chem. Soc*. **1998**, *120,* 3265-3266. Cited 1385 times.

2. **Zubarev, R. A**.; Fridriksson, E. K.; Horn, D. M.; Kelleher, N. L.; Kruger, N. A.; Carpenter, B. K.; McLafferty, F. W. *Electron Capture Dissociation for Structural Characterization of Multiply Charged Protein Cations, Anal. Chem.* **2000***, 72,* 563-573.. Cited 692 times.

3. **Zubarev, R. A.** *Reactions of Polypeptide Ions with Electrons in the Gas Phase, Mass Spectrom. Rev.* **2003,** *22***,** 57-77. Cited 292 times.

4. Pirmoradian, M.; Astorga-Wells, J.**; Zubarev, R. A.** *Multijunction Capillary Isoelectric Focusing Device Combined with Online Membrane-Assisted Buffer Exchanger Enables Isoelectric Point Fractionation of Intact Human Plasma Proteins for Biomarker Discovery. Anal Chem* **2015,** *87,* 11840-6.

5. Pirmoradian, M.; Aarsland, D.; **Zubarev, R. A**. *Isoelectric point region pI≈7.4 as a treasure island of abnormal proteoforms in blood, Discoveries* **2016**, *5,* e67. DOI: 10.15190/d.2016.14

Relevant previous projects or activities

Swedish Research Council, grant 621-2004-4897. Years: 2005-7, €200,000. "Towards complete structural characterization of proteins and protein complexes by tandem mass spectrometry with ion-electron reactions".

Swedish Research Council, project 621-2008-10. Year: 2008-10. €300,000. "A super-effective fragmentation method for proteomics."

Swedish Research Council, grant 2011-3699 for 2012-2015, €240,000, "Proteomics of neurodegenerative and inflammatory diseases using new methods of on-line isoelectric point separation.

VINNOVA, grant 2015-04208 for 2015-17, €195,000, "pI fractionation instrument".

Relevant infrastructure or equipment *(in relation to the present proposal)*

The Division of Physiological Chemistry I, headed by Roman Zubarev, includes the proteomics core facility PK/KI as well as a Molecular Biometry research group. The Division possesses the following high-end Fourier transform mass spectrometric equipment: Orbitrap LTQ Elite with ETD fragmentation technique (2008-2015), Orbitrap XL with ETD (2010), Orbitrap Q Exactive (2012), two Orbitrap Q Exactive Plus (both 2014) and Orbitrap Fusion (2014). All mass spectrometers are equipped with UPLC systems.

Main Tasks in TopSpec	**WP**
KI plays central role, integrating all the parts developed by other partners, creating software for seamless operation of the whole complex, providing samples, and analysing them using novel fragmentation techniques. KI is Coordinator and manager of the project (workpackage 9). KI is lead partner for WP2 (omnitrap development and testing), WP4 (Development and application of	2,3,4,5,6, 7,8,9

Coulomb explosion MS/MS technique) and will lead the exploitation, dissemination and communication activities in WP8.	

TopSpec - INTELLECTUAL PROPERTY (IP) ISSUES			
IP Owner/ inventor (s)	**Patent No.**	**Publication Year/ Title of Patent**	**Access Rights for TopSpec implementation**
German A. K., Bulat S. I., Zubarev R. A., Bondarenko P. V., Knysh A. N	SU1773461	1992/ Time-of-flight mass spectrometer	Access rights for TopSpec project purposes only, and only for the TopSpec project participants.
Zubarev R. A	EC H01J49/10; H01J49/42B2; US2004155180	2004// Mass spectrometry methods using electron capture by ions	
Zubarev R. A., Kjeldsen, F., Ivonin I., Silivra O. A	GB2405526	2005/ Electron-ion fragmentation reactions in multipolar radiofrequency fields	
Zubarev R. A., Baykut, G., Witt M	GB2414342	2005/Tandem mass spectrometry method	
Zubarev R. A., Savitski M. M., Nielsen M. L.	H01J49/02; G01N30/72; H01J49/02; G01N30/00; H01J49/16A. GB2429835	2007/ Tandem mass spectrometry with feedback control.	
Zubarev R. A., Misharin A.	US2007278402	2007/ Measuring cell for ion cyclotron resonance mass spectrometer	
Savitski M. M., Zubarev R. A.,	WO2009138179-A2, 2009.	2009/Mass spectrometry (MS)/MS data processing method for identifying precursor ion species from fragments forwards sample data identifying mass of particular precursor sample ion species and mass of multiple fragment ion species to comparator	
Zubarev R. A., Chingin K.	P126222GB00, 2012	2012/ Fragmentation of Biological Ions Activated with High-Energy Plasma Beam	
Zubarev R. A., Lyutvinskiy Y.	8388WO1/PCT	2012/ Method and apparatus for improved quantitation by mass spectrometry	
Zubarev R. A.	P9957IS00	2014/ Isotopic compositions of reactants for chemical, biochemical or biological reaction	

4.1.2 Participant 2

Participant number	**2**
Organization full name	Fasmatech Science and Technology SA
Organization Short name	FASM
Website	http://www.fasmatech.com

Description of the institution

Fasmatech Science and Technology SA/Greece (*www.fasmatech.com*) was founded in Athens, Greece in 2010, and an additional European office was established in Oxford, UK in Jan 2016. Fasmatech is a high technology SME focused on mass spectrometry and ion mobility instrumentation design and development for environmental and life sciences applications with a particular focus on diagnostics and high mass applications.

Fasmatech steady-growing R&D team currently employs an interdisciplinary team of 10 scientists (4 PhDs, 3 MScs) connecting many different branches of science including mass spectrometry and ion mobility instrumentation, ion optical theory, numerical methods for ion tracing, Monte Carlo methods, ion-molecule collision theory, computational gas dynamics, aeronautics engineering and flow visualization techniques, mechanical, electronics and software engineering.

Fasmatech has designed and installed unique prototype instrumentation platforms for several institutions across Europe including the University of Duisburg-Essen DE, the Paul Sabatier University of Toulouse FR, the University of Messina IT, the University of Rennes FR, the University of Maastricht NL, the University of Lille FR and the University of Rostock DE as well as a novel prototype platform that is currently under construction for the BESSY facility in Berlin. In addition, Fasmatech is successfully implementing a FET-RIA project (Viruscan – 731868).

Fasmatech's scientists are leaders in the field of low pressure gas dynamics utilizing advanced experimental tools and numerical methods to characterize and optimize mass spectrometry interfaces. Fasmatech has executed instrumentation contract services for major MS manufacturers (Shimadzu, Bruker and Thermo Fisher) and currently runs computational projects for Imperial College London, UK and the Department of Chemistry, University of Oxford, UK.

Fasmatech has been developing novel RF quadrupole ion trap instrumentation platforms with highly extended functionality and supported by high-end electronics, specifically suited to the purposes of the TopSpec project.

Key persons carrying out the research

Dr. Dimitris Papanastasiou (physicist, male), is one of the co-founders and technical director at Fasmatech. He is a leading expert in ion optics, ion traps, differential ion mobility spectrometry and time-of-flight mass analyzers. He has been involved in basic research, product R&D and several diverse prototype instrumentation development projects in mass and ion mobility spectrometry and has authored over 10 patent families, 10 peer review articles, 1 textbook chapter and over 50 conference papers. He is currently leading successfully a highly skilled team of scientists based in Athens providing customized instrumentation solutions to academic and research institutions across Europe and covering diverse areas of research.

Dr. Papanastasiou has deep knowledge on a variety of mass analyzers such as quadrupole ion traps, the orbitrap, time-of-flight analyzers including gridless reflectrons, ion optics, the use of electrodynamic lenses, ionization processes such as electrospray and laser desorption-ionization, collisional cooling techniques, vacuum designing, pressure measurements, and ion mobility related technology. Fasmatech's team has peer reviewed scientific publications in most of the afore mentioned areas of scientific research and development.

Dr. **Papanastasiou** obtained his BSC from the Environmental Science Dept at the University of the Aegean in Lesvos, Greece, on scintillation counters for radon monitoring. He holds an MSc degree on TOF MS and time-focusing theory and a PhD degree in TOF instrumentation development from MMU University in Manchester, UK. He carried out world-class research during post-doctoral training in New Mexico, USA on ion mobility-

mass spectrometry instrumentation. He has previously worked at the Shimadzu Research Laboratory (SRL) developing hybrid mass analyzers and differential mobility spectrometers. In August 2014, he received the Curt Brunnée Award at the International Mass Spectrometry Conference in Geneva for outstanding contributions to the development of the theoretical understanding of ion behaviors and implementation of this knowledge in new devices for ion optics and ion mobility. The award was primarily given for the successful demonstration of ion separation based on differential mobility properties in low pressure laminarized subsonic flows.

Relevant publications, products or services

D.Papanastasiou, et al; A Quadrupole Ion Trap Mass Spectrometer Coupled to a Vacuum Matrix-Assisted Laser Desorption Ionization Source, in R.E.March, J.F.J.Todd, (Eds), PRACTICAL ASPECTS OF TRAPPED ION MASS SPECTROMETRY, Vol. 4, Theory and Instrumentation, Chapter 19, Taylor & Francis, 2010.

S.Prasad, et al, "Computational Simulation of Ion Motion in FAIMS Through Combined Use of SIMION and Statistical Diffusion Simulation" Anal. Chem. 2009, 81(21), 8749.

D.Papanastasiou, et al, "Ion Thermalization Using Pressure Transients in a Quadrupole Ion Trap Coupled to a Vacuum Matrix-Assisted Laser Desorption Ionization Source and a Reflectron Time-Of-Flight Mass Analyzer". Rev. Sci. Instrum., 2008, 79, 055103. [selected for the Virtual Journal of Biological Physics Research., 2008, 15(10)].

D.Papanastasiou, H.Wollnik, G.Rico, F.Tadjimukhamedov, W.Mueller, G.A.Eiceman, "Differential Mobility Separation of Ions Using a Rectangular Asymmetric Waveform". J. Phys. Chem. A. 2008, 112, 3638-3645.

D.Papanastasiou, A.W.McMahon, "Correlated Phase Space Distributions of Ions in an Orthogonal Time-of-Flight Mass Spectrometer". Int. J. Mass Spectrom. 2006, 254, 20-27.

D.Papanastasiou, et al, "Dynamic Pressure Measurements during Pulsed Gas Introduction in a Quadrupole Ion Trap". Vacuum. 2006, 81, 446-452.

Publications/ Conferences

• ASMS 2015 (St. Louis, MO) Session: Ion/Molecule, Ion/Ion, Ion/Electron Interactions- 046. Ion-ion and ion-electron activation experiments in a novel linear ion trap, Dimitris Papanastasiou *et al*, Fasmatech Athens, GR

• ASMS 2016 (San Antonio, TX)- Accepted. A segmented Linear Quadrupole Ion Trap for Enhanced Activation and Storage, Dimitris Papanastasiou et al, Fasmatech Athens, GR

• IMSC2016 (Toronto, CA)- Submitted. Oral presentation / Title: Characterization of PAH Content and Distribution in Cosmic Dust Analogues, H. Sabbah *et al*, Paul Sabatier University of Toulouse.

• IMSC2016 (Toronto, CA)- Submitted. Poster presentation / Title: Exploring Collision Induced Dissociation of coronene cation C24H12+ in a Linear Quadrupole Ion Trap driven by Rectangular Voltage RF Waveforms. D. Papanastasiou *et al*, Fasmatech Athens, GR.

Relevant previous projects or activities

- Atmospheric – Vacuum Ion Interface for Orthogonal Extraction TOF Analyser Duisburg-Essen University, Institute for Combustion & Thermodynamics-Germany
- Particle Tracking Velocimetry measurements (PTV) of low pressure flows Bruker Daltonics- USA (content confidential)
- Particle Image Velocimetry measurements (PIV) in a proprietary capillary Thermofisher Scientific- USA (content confidential)
- Vacuum Differential Mobility Spectrometry (vDMS) development Shimadzu Corporation- Japan (content confidential)
- Gas Dynamics simulation, Faculty of Medicine, Department of Surgery & Cancer – London Imperial College

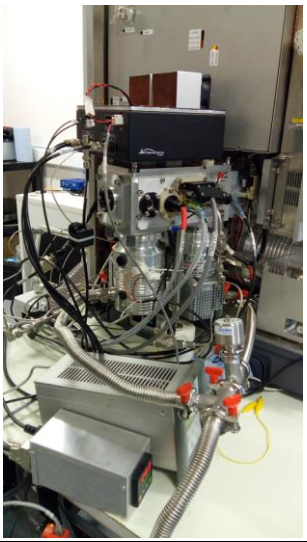
- Department of Chemistry - University of Oxford, *Charge state Enrichment by proton attachment*– Consultancy and instrumentation development project performed under service contract for Shimadzu Research Laboratory, UK 2013-2014 (content confidential).
- Department of Chemistry - University of Oxford, *Charge state Enrichment by proton attachment Laser desorption ionization linear quadrupole ion trap oTOF mass spectrometer* – Prototype instrumentation platform developed for IRAP, Paul Sabatier University of Toulouse, FR 2015-2016.

Relevant Patents

PATENT: Segmented Linear Quadrupole Ion Trap for Enhanced Activation and Storage, application U.S. Patent No. 9,978,578

Relevant infrastructure or equipment *(in relation to the present proposal)*

Early prototype of the Omni-trap coupled to a mass spectrometer



Main Tasks in TopSpec	**WP**
Fasmatech is the responsible PI for two work packages. WP1: Omni-trap & IM development and testing and adaptation to the Q-Exactive platform. WP3 Development and application of H-atom bombardment (HAB) MS/MS techniques. Their expertise in ion trapping methods, advanced experimentation with trapped ions and implementation of cutting edge activation-dissociation methods will be utilized in WPs 2 and 4. Fasmatech will play a very active role in exploitation processes in WP8, in terms of project and result exploitation.	1,2,3, 4,8,9

TopSpec - INTELLECTUAL PROPERTY (IP) ISSUES

IP Owner/ inventor (s)	**Patent No.**	**Publication Year/ Title of Patent**	**Access Rights for TopSpec implementation**
FT/ D. Papanastasiou, E. Raptakis	U.S. Patent No. 9,978,578	Filing date: 03 Feb 2016/ Segmented Linear Ion Trap for Enhanced Activation and Storage	High relevance for the TopSpec project. Access rights for TopSpec project purposes only, and only for the TopSpec project participants.

4.1.3 Participant 3

Participant number	**3**
Organization full name	Thermo Fisher Scientific
Organization Short name	TF
Website	www.thermofisher.com

Description of the institution

Thermo Fisher Scientific (TF) (Bremen) GmbH/Germany is a world market leader in mass spectrometry. Product lines include high-resolution mass spectrometers (MS) for organic analysis, isotope ratio analysis, elemental analysis, and life science. Following development of breakthrough Hybrid Linear Trap- Orbitrap MS of LTQ Orbitrap family over the last decade, recent products of the company include an Orbitrap-only MS (Exactive Plus, Exactive EMR instruments), Hybrid Quadrupole-Orbitrap MS (Q Exactive family currently containing Q Exactive, Q Exactive Plus, Q Exactive HF, Q Exactive Focus instruments). The team in Bremen is also involved in the launch of Tribrid Quadrupole-Linear Trap- Orbitrap instrument Orbitrap Fusion Lumos MS in 2015, Q Exactive HF-X in 2017 and Q Exactive UHMR in 2018 as well as in the development on nano- and micro-flow LC.

Key persons carrying out the research

Alexander A Makarov (AM) (male) (PhD) is Director of Global Research Life Science, Mass Spectrometry (Thermo Fisher Scientific (TFS), Germany, Bremen) and inventor of the Orbitrap mass analyzer. He is also professor of High Resolution Mass Spectrometry at Utrecht University (Netherlands). He has extensive expertise in instrument development and his invention of the Orbitrap mass analyzer in late 90s, changed the field of modern mass spectrometry.

He studied physics at Moscow Physics-Engineering Institute (MPEI) (Russia) where he obtained his pre-doctoral (Physics) and PhD (Physics and mathematics). After defending his PhD thesis in 1992 and joining the General Physics Institute of Russian Academy of Sciences, he became a post-doc in Warwick University where he got involved in tandem mass spectrometry. In 1996, Alexander joined a small high-tech company, HD Technologies, Manchester, UK where he started the concept of the Orbitrap mass analyzer. Proof-of-principle results were presented at the ASMS Conference and the term “Orbitrap analyzer” was used for the first time in 1999. Following the acquisition of HD Technologies by Thermo Corporation in 2000, Alexander concentrated his research on interfacing the analyzer to continuous rather than pulsed ion sources with the practical goal of incorporating an electrospray ion source.

This resulted in the commercial release of a new LTQ Orbitrap tandem mass spectrometer in 2005 to the enthusiastic acceptance of the mass spectrometry community, especially by proteomics and metabolomics researchers.

At TFS, he has continued to provide scientific guidance on the LTQ Orbitrap family of instruments (XL- 2007, ETD- 2008 Velos- 2009, Velos Pro and Elite -2011), Exactive family (Classic-2008, Plus- 2012, EMR- 2013), iCAP family (Q-2012, TQ- 2017), Q Exactive family (Classic- 2011, Plus- 2013, Focus and HF- 2014, HF-X- 2017, UHMR- 2018), Orbitrap Fusion family (2013, Lumos- 2015).

Pursuing the strategic goal of bringing Orbitrap technology into new analytical applications previously inaccessible to mass spectrometry, Dr. Makarov continues to drive improvements of the technology. This is illustrated by the introduction of a new generation of Orbitrap analyzers and enhanced Fourier transform algorithms).

Dr Makarov has over 90 peer reviewed publication and more than 100 issued US and 150 worldwide patents and several awards as a team director as well as a person.

For his role in the development of Orbitrap technology, Alexander received the Heinrich-Emmanuel Merck Award (2007), Award for Distinguished Contribution in Mass Spectrometry of American Society for Mass Spectrometry (2008), Gold Award of the Russian Society for Mass Spectrometry (2007), Curt Brunnee Research Award (2009), Science and Technology Award of the Human Proteome Organization (Geneve 2011), Thomson medal of International Society for Mass Spectrometry (Kyoto 2012) and others.

Team awards include Best New Spectroscopy Product of 2015 from SelectScience for Q Exactive GC instrument (Pittcon 2016), R&D 100 Award for Q Exactive Focus instrument, Best New Spectroscopy Product of 2013 from SelectScience for Thermo Scientific, Orbitrap Fusion Tribrid Mass Spectrometer (Pittcon 2014), Gold Editors Award for LTQ Orbitrap instrument, Pittcon (Orlando 2006) and others.

Relevant publications, products or services

- J. Gault, J. Donlan, I. Liko, J. Hopper, Kallol Gupta, N. Housden, W. Struwe, M. Marty, T. Mize,C. Bechara, Ya Zhu, Beili Wu, C. Kleanthous, M. Belov, E. Damoc, A. Makarov, C. Robinson. “High-resolution mass spectrometry of small molecules bound to membrane proteins”. Nature Methods, 2016, doi:10.1038/nmeth.3771
- O. Skinner, P. Havugimana, N. Haverland, L. Fornelli, B. Early, J. Greer, R. Fellers,K.Durbin, L. Do Vale, R. Melani, H. Seckler, M. Nelp, M. Belov, S. Horning, A. Makarov, R. LeDuc, V. Bandarian, P. Compton, N. Kelleher. “An informatic framework for decoding protein complexes by top-down mass spectrometry”. Nature Methods, 2016, 13: 237–240. doi:10.1038/nmeth.3731
- Rose RJ, Damoc E, Denisov E, Makarov A, Heck AJR (2012) High-sensitivity Orbitrap mass analysis of intact macromolecular assemblies. Nature Methods 9, 1084-1086
- J. Olsen, B. Macek, O. Lange, A. Makarov, S. Horning, and M. Mann (2007). “Higher-energy C-trap dissociation for peptide modification analysis”, Nature Methods 4 (9), 709-712.
- Makarov (2000). Electrostatic axially harmonic orbital trapping: a high-performance technique of mass analysis, Anal. Chem. 72, 1156-1162.

Relevant previous projects or activities

HR/AM R&D projects for Life Science in the Bremen factory, budget >€13 Mio/year

PROSPECTS (EU, Collaborative Project, HEALTH F4 2008 001648) 4/2008 – 3/2013

MSMED (EU, H2020-FETOPEN-2014-2015 No. 686547) 1/2016-12/2019

Relevant Patents

M. Belov, "Method and Apparatus for Mass Spectrometry of Macromolecular Complexes", US2015340213

D. Grinfeld et al, "Ion guide", US9536722, WO2014048837.

A. Makarov, E. Denisov, E. Damoc, "Method and Analyser for Analysing Ions Having a High Mass-to-charge Ratio", US8791409.

J.-P. Hauschild, A. Kholomeev, E. Denisov, et al. "Mass analyser", with US2014191122, US8841604, WO2012152950.

J.-P.Hauschild, O. Lange, A.Makarov, et al "Method and apparatus for mass analysis", US2014061460, WO2012160001.

E. Denisov, A. Kholomeev, J.-P.Hauschild et al. "Mass analyser", WO2012152950.

E. Denisov, W. Balschun, D. Nolting, et al. "Collision Cell", WO2009147391, US9396919, US9245723.

A. Kholomeev, A.Makarov, "Mass Spectrometer", with WO2009144469.

A.Makarov, "Vacuum Pump Or Vacuum Apparatus Having A Vacuum Pump", WO2008104314.

Grinfeld D.; Monastyrskiy M., A.Makarov, "Ion trap", WO2008081334, US8017909.

A.Makarov, E.Denisov, G. Jung, et al. "Improvements in an electrostatic trap", WO2006129109.

A.Makarov, E. Denisov, G. Jung, et al "Improvements related to mass spectrometry", WO2006103412.

A.Makarov, "Improvements related to mass spectrometer", WO2006103448.

A.Makarov, E. Denisov, G. Jung, et al "Improvements related to ion trapping", WO2006103445.

A. Kholomeev, E. Denisov, A.Makarov, "RF power supply for a mass spectrometer", WO05124821.

A.Makarov, M. Hardman, J. Schwartz, M. Senko. "Mass spectrometry method and apparatus", US6872938, US6995364, US6998609, US7265344, US7425699, WO02078046.

Access rights for TopSpec project purposes only, and only for the TopSpec project participants.

Relevant infrastructure or equipment *(in relation to the present proposal)*

The research and development team represented by Alexander Makarov has a unique track record in mass spectrometry innovation which is best represented by introduction of the ground-breaking Orbitrap analyser, first as a part of hybrid LTQ Orbitrap and then as a basis for two further instrument families, (Q) Exactive and Orbitrap Fusion. Culture of continuous innovation enabled a number of significant technological advances such as C-trap, HCD fragmentation cell, high- and ultra-high field compact Orbitrap in instrumentation, parallel filling/detection, spectra multiplexing in instrument control, enhanced Fourier transform in signal processing, Proteome Discoverer and Compound Discoverer for HR/AM applications. These innovations enforced significant instrument improvements every 2 years over the last decade and turned Orbitrap platform into the major workhorse for most of proteomic research. The group has all skills and equipment for complete development of commercial instruments with control and application software and has access to all generations of Orbitrap instruments as well as associated liquid separations and ion sources.

Latest Orbitrap instruments, Q Exactive HF-X (to be used in this project) and Orbitrap Fusion Lumos MS.

Main Tasks in TopSpec	**WP**
Thermo Fisher Scientific will lead WP 6 (Modification of the Orbitrap mass spectrometer) and will provide a State of the art Q Exactive instrument to the project which will be integrated with the Omni-trap. Thermo Fisher Scientific will also work on ion optics development, instrument control software and will provide expertise in analysis of intact proteins both under native and denaturing conditions. Thermo Fisher Scientific will support the dissemination and communication activities in WP 8 and will collaborate with both academic and the SME partners in the exploitation tasks of that WP.	1,2,3,5,6,8,9

4.1.4 Participant 4

SpectroSwiss

Participant number	**4**
Organization full name	Spectroswiss
Organization Short name	SPS
Website	www.spectroswiss.ch

Description of the organisation

Spectroswiss Sarl (GmbH) is an innovation-driven SME founded in 2014 and directed by Dr. Yury Tsybin as a spin-out from his Biomolecular Mass Spectrometry Laboratory at the Ecole Polytechnique Federale de Lausanne (EPFL) in Switzerland. Spectroswiss is active in hardware and software research and development for high-performance Fourier transform mass spectrometry (FTMS), advancing excellence in science via EU and Swiss-funded research, providing consulting and training services in FTMS. Since 2017 Spectroswiss first products, a high-performance data acquisition system (FTMS Booster) and FTMS data processing framework (Peak-by-Peak) are commercially available. The customers include analytical departments in life and environmental sciences industry and academia. Spectroswiss consolidates developments in FTMS data processing originating from research institutions in Europe. Spectroswiss partners and customers are distributed worldwide, with the majority being in the EU and in the USA. Currently active EU projects include an ERC Proof of Concept action and a Eurostars project (a 3 year project to start on July 1st 2018). Both these projects relate to the FTMS domain of activities, but without an overlap with the current project proposal.

The core team of Spectroswiss consists of 3-4 FTEs experts in FTMS with a complementary background. Spectroswiss headquarters are located at the vibrant EPFL Innovation Park in Lausanne, Switzerland. Spectroswiss scientists actively participate in the scientific activities of FTMS community. In a recognition of achievements by Dr. Yury Tsybin and his research groups at EPFL (2006-2014) and at Spectroswiss, he has been awarded the prestigious Curt Brunnée Award of the International Mass Spectrometry Society in 2016.

Profile match to TopSpec project: extensive experience in the related FTMS method development and applications, including top-down and middle-down mass spectrometry development with consecutive applications for antibody structure analysis; direct use of innovations and know-how for achieving project objectives, including in data acquisition electronics and signal processing development; securing related IP required for future envisioned product commercialization and results dissemination; project management experience and a broad professional network matching the scope and the targeted applications of TopSpec.

Key persons carrying out the research

Dr. Yury O. Tsybin (male) received his PhD degree in ion physics in 2004 from Uppsala University, Sweden under supervision of Prof. Per Hakansson. For the next 2 years Yury was a postdoctoral research associate with Prof. Alan G. Marshall at the National High Magnetic Field Laboratory in the USA. From 2006 to 2014 Yury was an assistant professor of physical and bioanalytical chemistry at the Ecole Polytechnique Fédérale de Lausanne (EPFL) in Switzerland where he established and headed the Biomolecular Mass Spectrometry Laboratory and served as a Director of the Mass Spectrometry Service Facility. In 2014 he founded an EPFL spin-off company, Spectroswiss Sàrl, which he is directing since then. Owing to his outstanding performance at these management positions and an extensive expertise of managing EU actions, Yury Tsybin is well prepared to manage and direct any workprograms of the TopSpec project.

Research interests of Yury are around the high-performance mass spectrometry and tandem mass spectrometry method and technique development with subsequent applications for in-depth analysis of biological and environmental samples. Particular instrumentation interests are in FTMS fundamentals, ion motion and ion detection, electron injection systems for ion fragmentation in the gas phase, data acquisition and signal processing. Application interests are in structural analysis of monoclonal antibodies and their mixtures for improving drug discovery and quality control technologies; middle-down and top-down proteomics development. Yury has authored more than 90 papers cited more than 2500 times; h-index is 28 (Scopus) or 31 (Google Scholar) and owns three patents. He has supervised 6 PhD students (graduated from EPFL) and co-supervised 1 PhD student (graduated from University of Geneva).

In 2011 Tsybin received the European Research Council (ERC) Starting Grant to pioneer and develop the super-resolution mass spectrometry for the applications in health and environmental areas. Project achievements include implementation and development of super-resolution signal processing methods for mass spectrometry-based qualitative and quantitative proteomics and metabolomics and novel mass analyzers for improved high-performance FT-ICR MS. In relation to the parent ERC StG, Yury has obtained two ERC Proof of Concept Grants (one completed and one on-going) to investigate the feasibility of a practical/commercial implementation of innovations resulting from the ERC StG research. For the outstanding contributions to science and education, in 2012 Tsybin received a European Young Chemist Award, in 2014 the SGMS award of the Swiss Group for Mass Spectrometry, and in 2016 a prestigious Curt Brunnée Award from the International Mass Spectrometry Foundation. Together with Julia Chamot-Rooke (lead for IP partner of TopSpec) he is also a founding member of the International Consortium for Top-Down Proteomics: http://www.topdownproteomics.org

The main (young) project participant from Spectroswiss, is **Dr. Anton Kozhinov** – a graduate of a PhD program in Chemistry at the EPFL (under a supervision of Prof. Yury Tsybin) in 2015. Since graduation, Anton has been a lead scientist at Spectroswiss. His main fields of expertise are data acquisition electronics and data processing algorithms. Anton is an extremely talented and exceptionally well-educated young scientist. His expertise will be instrumental in achieving the ambitious goals of TopSpec. The work of Dr. Kozhinov will be supported by another Spectroswiss team-member, **Dr. Konstantin Nagornov**, a post-doctoral scientist at Spectroswiss more experienced in practical mass spectrometry. Previously, Konstantin was a postdoc at the Biomolecular Mass Spectrometry Laboratory at EPFL (headed by Prof. Yury Tsybin). His main expertise is hands-on experimental work with ultra-high resolution mass spectrometry platforms and workflows, as well as data processing and data analysis approaches. Owing to the complementary background and expertise of the two Spectroswiss experienced scientists, their joint participation in the TopSpec activities will be highly recommended as beneficial for the project. Other Spectroswiss team members will be involved as needed.

Overall, Yury Tsybin and his team at Spectroswiss, their expertise and research interests perfectly match the objectives, methodology, and scale of TopSpec.

Relevant publications, products or services

- Hardware product: High-performance data acquisition system, FTMS Booster
- Software product: FTMS data processing and data analysis framework, Peak-by-Peak
- Fornelli Luca, Daniel Ayoub, Konstantin Aizikov, Xiaowen Liu, Eugen Damoc, Pavel A. Pevzner, Alexander Makarov, Alain Beck, and Tsybin Yury O. Top-down analysis of immunoglobulin G isotypes 1 and 2 with electron transfer dissociation on a high-field Orbitrap mass spectrometer. *Journal of Proteomics*, (2017) 159, 67–76.
- Aushev Tagir, Kozhinov Anton N., Tsybin Yury O. Least-squares fitting of time-domain signals in Fourier transform mass spectrometry. *Journal of the American Society for Mass Spectrometry* (2014) 25(7) 1263-1273
- Fornelli Luca, Ayoub Daniel, Aizikov Konstantin, Beck Alain, Tsybin Yury O. Middle-down analysis of monoclonal antibodies with electron transfer dissociation Orbitrap FTMS. *Analytical Chemistry* (2014) 86, 3005-3012

- Srzentic Kristina, Fornelli Luca, Laskay Unige A., Monod Michel, Beck Alain, Ayoub Daniel, Tsybin Yury O. Advantages of extended bottom-up proteomics using Sap9 for analysis of monoclonal antibodies. *Analytical Chemistry*, 2014, 86, 9945−9953
- Fornelli Luca, Parra Julien, Hartmer Ralf, Stoermer Carsten, Lubeck Markus, Tsybin Yury O. Top-down analysis of 30–80 kDa proteins by electron transfer dissociation time-of-flight mass spectrometry. *Analytical Bioanalytical Chemistry* (2013) 26, 8505-8514
- Fornelli Luca, Damoc Eugen, Thomas Paul M., Kelleher Neil L., Aizikov Konstantin, Denisov Eduard, Makarov Alexander, and Tsybin Yury O. Top-down analysis of monoclonal antibody IgG1 by electron transfer dissociation Orbitrap FTMS. *Molecular and Cellular Proteomics* (2012) 11(12):1758-67
- Kozhinov Anton N., Tsybin Yury O. Filter diagonalization method-based mass spectrometry for molecular and macromolecular structure analysis. *Analytical Chemistry* (2012) 84, 2850-2856
- Tsybin Yury O., Fornelli Luca, Parra Julien, Stoermeer Carsten, Luebeck Markus, Nallet Sophie, Wurm Florian M., Hartmer Ralf. Structural analysis of intact monoclonal antibodies by electron transfer dissociation mass spectrometry. *Analytical Chemistry* (2011), 83 (23), 8919–8927

Relevant previous projects or activities

- 2017-2018: ERC Proof of Concept Grant (PI), " Precision mass spectrometry to leverage applications in life and environmental sciences". HI: Spectroswiss. Total amount: 150’000 Euros
- 2011-2016: ERC Starting Grant (PI), "Super resolution mass spectrometry for health and sustainability". HI: EPFL (Periods 1-3) and Spectroswiss (Periods 4-5). Total amount: 1'425'731 Euros
- 2015-2016: ERC Proof of Concept Grant (PI), "Advanced signal processing of time-domain data in mass spectrometry to leverage life sciences". HI: Spectroswiss. Total amount: 150’000 Euros
- 2013-2016: Swiss National Science Foundation (PI), Independent Basic Research Grant. "Advancing mass spectrometry-based protein structure analysis at the proteoform level for comprehensive description of biological systems". Total amount 175’000 Euros
- 2009-2013: Swiss National Science Foundation (PI), Independent Basic Research Grant. "Understanding energy absorption & relaxation pathways in dehydrated biomolecular systems upon electron capture & transfer dissociation for probing peptide and protein structures and interactions". Total amount: 342’000 Euros

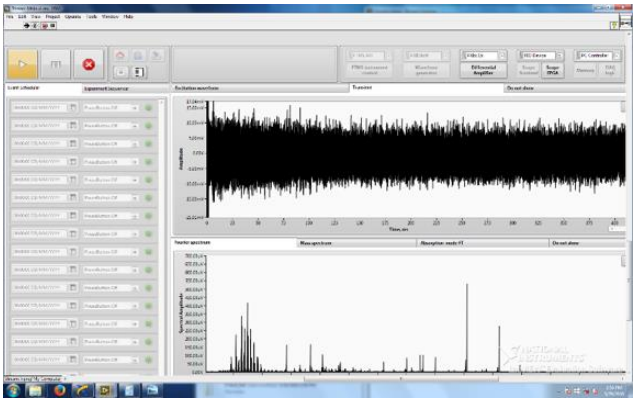
Relevant Patents

International Patent Application n° PCT/IB2016/051887 filed on April 1st, 2016

Title: "Data acquisition apparatus and methods for mass spectrometry". Applicant: Spectroswiss Sàrl. Inventors: Anton Kozhinov, Yury Tsybin, Konstantin Nagornov.

Relevant infrastructure or equipment

- fully equipped offices with the required computational infrastructure (includes multicore workstations with high-performance graphic cards)
- equipped electronics laboratory for data acquisition systems development
- equipped wet-lab for sample preparation
- LTQ Orbitrap XL FTMS instrument for method and technique development



Main Tasks in TopSpec	WP
Spectroswiss will lead the software development and data processing tasks in workpackage 7: Advanced signal processing and data analysis software production. A hardware-software combination will increase the sensitivity of top-down analysis. Spectroswiss will also be actively involved in exploitation activities (WP8).	7,8,9

TopSpec - INTELLECTUAL PROPERTY (IP) ISSUES			
IP Owner/ inventor (s)	**Patent No.**	**Publication Year/ Title of Patent**	**Access Rights for TopSpec implementation**
Spectroswiss Sàrl/ Anton Kozhinov, Yury Tsybin, Konstantin Nagornov	International Patent Application n° PCT/IB2016/051887 filed on April 1st, 2016	2016/ Data acquisition apparatus and methods for mass spectrometry	Access rights for TopSpec project purposes only, and only for the TopSpec project participants.

4.1.5 Participant 5

Participant number	**5**
Organization full name	Biomotif AB
Organization Short name	BM
Website	biomotif.com

biomotif
Capturing the essence of life

Description of the organisation

Biomotif is a Swedish SME formed in 2005 by Thorleif Lavold. The company develops new front-end instrumentation for mass spectrometry. Its main objective is to simplify methods and instrumentation by integrating novel technology with next generation mass spectrometer systems.

Biomotif has developed different analytical devices like, ElectroCapture, HX-IA and pI Trap Instrument™ for advanced proteomics and interactomics studies. The technology is based on several inventions including ElectroCapture, Membrane-assisted H/DX, isoelectric focussing and on-line pI separation of proteins and peptides.

The HX-IA Instrument™ provides a better understanding of biomarker targets, their structure/conformation, dynamics and molecular interactions with potential drug candidates in the liquid phase.

The pI Trap Instrument™ is the latest product for truly Gel-Free, MS-Compatible, pI-Fractionation of complex biological samples.

Biomotif also provides a number of contract analysis services using the HX-IA Instrument and mass spectrometry for drug development and target validation including:

Drug Discovery – Small Molecule and Antibody –

Protein-antibody epitope mapping and identification

Protein-protein or protein-peptide interface mapping

Bioequivalence studies

Patent submission/protection information

H/DX screening for structure-based drug design

Structural Biology

Identification of disordered regions in protein constructs that prevent crystallization

Identifying the effect of mutations on protein stability

Complementary data to x-ray and NMR data

Key persons carrying out the research

Dr. Juan Astorga Wells, Scientific manager at Biomotif, *is a* Pharmacist and holds a PhD in Medical Biochemistry. Role as the PI in this project.

Juan is a promising young scientist with extensive experience in instrument building and testing, with special knowledge of in the combination of capillary isoelectric focusing, capillary electrophoresis and membrane-based capillary electrophoresis with mass spectrometry for the analysis of proteins and peptides. Juan is co-author on 24 peer-reviewed publications related to protein analysis by mass spectrometry

Thorleif Lavold (TL) (male) *is a businessman and entrepreneur with focus on* mass spectrometry and life science applications. In addition to Biomotif AB (2005), he has started Zula Diagnostics AB (2012) and HDXperts AB (2014).

Thorleif has more than 25 years of experience in the sales, marketing and launching of new mass spectrometers, and a successful background in multiple instrumentation companies: VG Instruments Ltd., Fisons Plc, Micromass AB (CEO) and Waters AB (Business Development). He was Sales and Managing Director in Micromass, where he sold the two first Q-Tof hybrid mass spectrometers in the world to Astra Zeneca and Pharmacia.

In 2005, Thorleif founded Biomotif, and two patents were purchased related to the ElectroCapture technology. Biomotif holds patents in the field of ElectroCapture, H/D Exchange MS (licensed to HDXperts AB) and pI Trap technology.

In 2014 Thorleif started HDXperts, a CRO offering Fee for service for molecular interactions and Epitope Mapping.

Thorleif has been Project leader for the three EUROSTARS projects; E4343, E9636 and E9792.

Relevant previous projects or activities

Two EUROSTARS funded projects have been completed, and one is ongoing: ElectroCapture (E4343), Findtheneedleinthehaystack (E9636) and pI Fractionator (E9792- on-going).

These projects have led to the prototype products in the field of proteomics to be developed in the TopSpec project. The IP and knowledge from the Eurostars projects has matured into the pI Fractionator initiative. Findtheneedleinthehaystack and pI Fractionator (on-going). These projects have led to prototype products in the field of proteomics.

Relevant Patents

Biomotif has granted patents (IPR; No. US 7,731,827 & US 8,237,117) on ElectroCapture technology.

Biomotif has also filed a provisional patent for the more complex pI Fractionator technology.

The patents relate to the use and manufacture of different configuration of the pI Trap Cell (Method and Apparatus), as well as the combination of such designs with other analytical techniques such as CZE. The novel manufacture allows better performance and robustness, as well as facilitating the manufacture its usability by the end-user. The novel design is especially suited for miniaturized separation techniques such as CZE. The successful combination of pI-Trap separations (based on isoelectric focusing) and CZE (based on electrophoretic mobility) provides improved separation power over standard proteomic-separation (nano-LC), and solves the inherent loading-capacity problems of using CZE alone. o ignored

Additionally, the patent also describes a microfluidic-chip design tailored to facilitate the collection of fractions via a parallelized-fluidic channel design that is built into the pI trap separation channel that will provide improved separations and facilitated automated fraction collection.

Relevant infrastructure or equipment

Orbitrap MS

HX-IA instrumentation for HD-Exchange MS

HPLC equipment

pI Trap instrumentation.

The Biomotif pI-TRAP System is a gel-free, 100 microgram scale, pI-fractionation solution for peptides & proteins. Delivering up to 30 fractions per hour to leverage LC-MS-MS based proteomics workflows.

5 Relevant publications, products or services

- Chingin, K.; Astorga-Wells, J.; Pirmoradian Najafabadi, M.; Lavold, T.; Zubarev, R. A. Separation of polypeptides by isoelectric point focusing in electrospray-friendly solution using multiple-junction capillary fractionator, Anal. Chem. 2012, 84, 6856-6862.http://www.ncbi.nlm.nih.gov/pubmed/22779778
- Pirmoradian, M.; Zhang, B.; Chingin, K.; Astorga-Wells, J.; Zubarev, R. A. Membrane-assisted isoelectric focusing device as a micro-preparative fractionator for two-dimensional shotgun proteomics, Anal. Chem, 2014, 86, 5728-5732. http://pubs.acs.org/doi/abs/10.1021/ac404180e
- Pirmoradian, M.; Astorga-Wells, J.; Zubarev, R. A. Multijunction Capillary Isoelectric Focusing Device Combined with Online Membrane-Assisted Buffer Exchanger Enables Isoelectric Point Fractionation of Intact Human Plasma Proteins for Biomarker Discovery. Anal Chem 2015, 87, DOI: 10.1021/acs.analchem.5b03344.
  http://www.ncbi.nlm.nih.gov/pubmed/26531800
- Membrane protein identifications by mass spectrometry using electrocapture-based separation as part of a two-dimensional fractionation system. Astorga-Wells J, Tryggvason S, Vollmer S, Alvelius G, Palmberg C, Jörnvall H. (2008) Anal. Biochem. 1;381(1):33-42.
- Microfluidic electrocapture-assisted mass spectrometry of membrane-associated polypeptides. Shariatgorji M, Astorga-Wells J, Jornvall H, Ilag LL. Anal. Chem. 2008 Sep 15;80(18):7116-20. 2008

Main Tasks in TopSpec	**WP**

Biomotif is lead PI for Workpackage 3, and responsible for design and building of the CIEF system, its interface with Orbi MS. Biomotif will also adaption the pI-Trap as a first dimension of separation in the TopSpec, and its connection to the UV detector.	4,5,8,9

TopSpec - INTELLECTUAL PROPERTY (IP) ISSUES			
IP Owner/ inventor (s)	**Patent No.**	**Publication Year/ Title of Patent**	**Access Rights for TopSpec implementation**
Biomotif	US 7,731,827 & US 8,237	2008/ ElectroCapture technology.	Access rights for TopSpec project purposes only, and only for the TopSpec project participants
Biomotif	Provisional	2017/ pI Fractionator technology	Access rights for TopSpec project purposes only, and only for the TopSpec project participants

4.1.6 Participant 6

Participant number	**6**
Organization full name	**THE NOTTINGHAM TRENT UNIVERSITY**
Organization Short name	TNTU
Website	www.ntu.ac.uk

NOTTINGHAM TRENT UNIVERSITY

Description of the Organisation

Nottingham Trent University (TNTU) is a public university located in the UK city of Nottingham and is celebrating the 175th anniversary of its founding institution in 2018. TNTU was named both the UK University of the Year and Modern University of the Year in 2017. Research at TNTU is world-class and, in recognition of that, we were awarded in 2015 with the highest national honour for a UK university – the Queen's Anniversary Prize.

Nottingham Tent University currently has almost 30,000 students, of whom more than 5,700 are postgraduate level. The university is composed of three colleges (Business, Law and Social Sciences; Art & Design, Architecture Design and Humanities; and Science and Technology) and nine schools. The Department of Chemistry sits in the School of Science and Technology, in the College of Science and Technology, and is based on TNTU's Clifton science Campus in the south of the city.

The Trent Integrated Mass Spectrometry (TIMS) joint research grouping was created in the School of Science and Technology in 2017 and crosses the boundaries between the Departments of Chemistry, Biosciences and Forensics. The aim of TIMS is to build on TNTU's historic strength in the area of mass spectrometry and drive future growth. TIMS is divided into 3 directorates: Mass Spectrometry Systems Development, Biomedical Applications and Forensic Applications.

Key persons carrying out the research

David P A Kilgour (DK) (male) (PhD) is a Senior Lecturer in Analytical Chemistry and a Director the Trent Integrated Mass Spectrometry Facilities (TIMS) at Nottingham Trent University (TNTU), in the UK.

Dr Kilgour studied Geochemistry at the University of St Andrews and went on to complete his PhD in The Development and Applications of Instrumental Chemical Analysis at the University of Edinburgh. He continued in Edinburgh and, during his postdoctoral studies, developed a data station and software for a prototype Orbitrap system, in collaboration with a team including AM, at TFS.

In 2003 he moved to join the UK Ministry of Defence (MoD), rising to the level of Principal Scientist, and Scientific Advisor to the Assistant Chief of the Defence Staff. During his time with the MoD he acted as Technical Lead for mass spectrometry projects and led a wide variety of programmes developing analytical instrumentation for security applications. One of these programmes was the joint UK/US Handheld Mass Spectrometer (HHMS) program that included directing research and graduate students at the universities of Lyon (France), Innsbruck (Austria) and Purdue (USA).

In 2011 DK moved to Warwick University as a Senior Research Fellow and was then invited to join the faculty at the University of Maryland Baltimore, as a Research Associate Professor, in 2013. In 2015 he was offered a position as a Senior Lecturer in Analytical Chemistry at Nottingham Trent University where he is continuing his research in mass spectrometry systems development.

Dr Kilgour undertakes research mainly in the areas of novel ion sources, mass analyzer developments and data processing algorithms. Amongst other research, he developed the Autophaser algorithm that uses a genetic

algorithm to optimally phase correct Fourier transform mass spectrometry (FTMS, a class that includes the Orbitrap) data, allowing the presentation of the data in absorption mode. He also developed the asymmetric apodization method (termed the "Kilgour" apodization in Bruker software), also for absorption mode FTMS. In 2017, he developed the AutoPiquer algorithm – a high confidence peak detection method that is proving highly beneficial for top-down protein mass spectrometry applications.

Relevant publications, products or services

Campuzano ID, Netirojjanakul C, Nshanian M, Lippens JL, Kilgour DP, Van Orden S, Loo JA. Native-MS Analysis of Monoclonal Antibody Conjugates by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. Analytical Chemistry. 2017 18;90(1):745-51

Kilgour DPA, Hughes S, Kilgour SL, Mackay CL, Palmblad M, Tran BQ, Goo YA, Ernst RK, Clarke DJ, Goodlett DR. Autopiquer-a Robust and Reliable Peak Detection Algorithm for Mass Spectrometry. J. Am. Soc. Mass Spectrom. 2017 Feb 1;28(2):253-62.

Tran BQ, Barton C, Feng J, Sandjong A, Yoon SH, Awasthi S, Liang T, Khan MM, Kilgour DP, Goodlett DR, Goo YA. Comprehensive glycosylation profiling of IgG and IgG-fusion proteins by top-down MS with multiple fragmentation techniques. Journal of Proteomics. 2016, 134, 93-101.

Kilgour DPA, Van Orden, S. Absorption mode FT-MS with no baseline correction using a novel asymmetric apodization function. Rapid Commun. Mass Spectrom. 2015, 29, 1009-1018.

Kilgour DPA, Neal MJ, Soulby AJ, O'Connor PB. Improved optimization of the Fourier transform ion cyclotron resonance mass spectrometry phase correction function using a genetic algorithm. Rapid Commun. Mass Spectrom., 2013, 27(17), 1977-1982.

Relevant previous projects or activities

"Protection Against Gram-Negative Sepsis Conferred by Lipid A-Based Structural Variants."; $100k; 2016; ongoing; NIH. DK is a named collaborator on this grant. PIs are Profs Goodlett and Ernst at UMB. Total grant funding is $100k. DK input will be the development of robust peak picking algorithms and the development of improved algorithms for automated structural characterization of lipids.

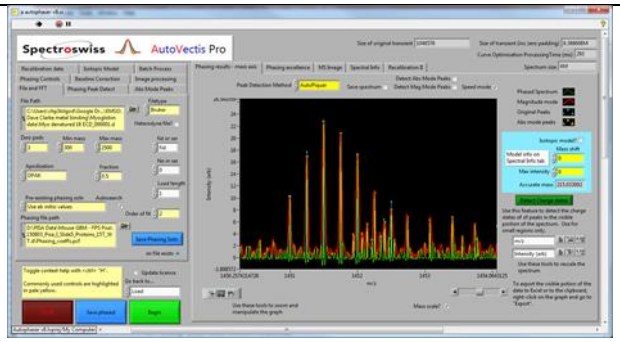
"High-Throughput Identification of Biological Toxins in Complex Matrices."; $1.4M; ongoing; US DoD funding through JSTO. DK is a named collaborator. PIs are Raymond Sullivan and Jon Oyler.

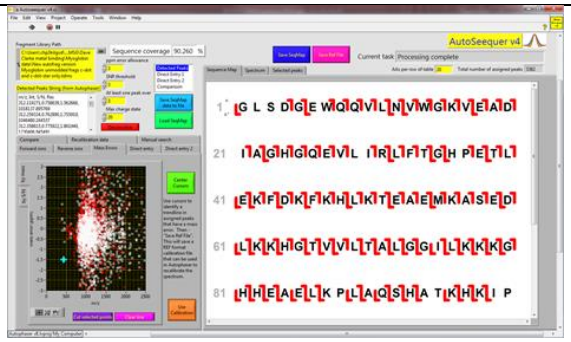
Software product: Absorption mode FTMS data processing software suite: AutoVectis.

Relevant infrastructure or equipment *(in relation to the present proposal)*

The Trent Integrated Mass Spectrometry Facilities (TIMS) at Nottingham Trent University (TNTU) provide the relevant specialist mass spectrometry facilities including:

- Dedicated mass spectrometry instrumentation laboratories (inc. Waters Xevo G2-XS QTOF, Sciex TripleTOF 6600 and 5600+ and Bruker Ultraflex MS systems)
- Sample preparation facilities – including clinical proteomics laboratory
- Specialist mass spectrometry systems development laboratory
- Computational support and infrastructure for mass spectrometry data processing and algorithm development, including 2 high performance computing clusters.





Main Tasks in TopSpec	WP
TNTU will engage mainly with the software development and data processing tasks in work package 7: Signal detection and Data processing, developing advanced algorithms for deriving protein sequence information from hardware deconvolved top-down fragmentation datasets including methods for software deconvolution, absorption mode processing, advanced peak detection and statistical spectral metrics of confidence. TNTU will also have a significant input into exploitation activities (WP8).	2,3,4, 5,7,8, 9

4.1.7 Participant 7

Participant number	**7**
Organization full name	Institut Pasteur
Organization Short name	IP
Website	www.pasteur.fr

Description of the institution

The **Institut Pasteur** is a private, state-approved non-profit foundation for biomedical research which was established in 1887 by Louis Pasteur and hosts some 2,400 people (scientists, engineers, technicians and administrative staff) of over 60 nationalities.

Its mission is built upon three cornerstones:

Research: 10 research departments divided into 130 research units bringing together multidisciplinary teams of scientists work at the very forefront of infectious diseases research, and are also dedicated to immunology, molecular biology, neurosciences, development biology, stem cells, genetics and genomics.

Teaching: The Institut Pasteur **Teaching Centre** offers 27 courses attended each year by about 500 students from all over the world and is also a **training centre** for young scientists (Master or PhD fellows). Courses cover three main areas of study: Mechanisms of living organisms, Biology of microorganisms and Epidemiology and Public Health.

Public Health: The Institut Pasteur's public health mission is to promote the transfer of scientific discoveries made in its research laboratories to human health applications. It is actively involved clinical research projects aimed at developing innovative therapies. As a microbiological observatory for communicable diseases, Institut Pasteur coordinates epidemiological surveillance of diseases throughout France *via* the **15 National Reference Centres** (CNR) hosted by its laboratories. These centres support health authorities in the areas of diagnosis, epidemiological surveillance, and research. Of the 15 centres, seven are also **World Health Organization Collaborating Centres** (WHOCCs) tasked with the same duties in the international arena.

The Institut Pasteur is also at the centre of a unique **international network of 32 institutes**, stretching across all five continents and all affiliated in partnerships.

The **Mass Spectrometry for Biology Laboratory** is mixed Institut Pasteur and CNRS laboratory. It includes a research group, dedicated to the development of structural proteomics (top-down proteomics, cross-linking combined to MS, native MS and HX MS) and a proteomics platform (bottom-up proteomics).

Key persons carrying out the research

Julia Chamot-Rooke (JCR) (female) (PhD) received a Ph.D. in Analytical Chemistry from Pierre et Marie Curie University (Paris) in 1996. Following post-doctoral positions at the University of Copenhagen and the University of Amsterdam, she joined the CNRS as a junior scientist in 1998. She worked in the Department of Chemistry at the Ecole Polytechnique until 2012 where she mainly used FT-ICR (Fourier Transform Ion Cyclotron Resonance) Mass Spectrometry to study peptides and small proteins. She was appointed a senior scientist by the CNRS in 2010. In September 2012 she moved to the Institut Pasteur Paris, as head of the newly created Structural Mass Spectrometry and Proteomics Lab. Her research focuses on the development of new methodologies in structural proteomics, such as top-down proteomics, for the analysis of proteins involved in infectious diseases. Julia Chamot-Rooke has published 69 papers in peer-reviewed journals (h-index: 22, sum of times cited without self-citation: 1320), has been invited in 21 international conferences/workshops in the last

three years and has been coordinator of several multidisciplinary projects funded by the French Research Agency and H2020. Julia Chamot-Rooke is a past president [2008-09] of the French Society of Mass Spectrometry (SFSM) and the French representative of the International Mass Spectrometry Foundation. She is also a founding member of the International Consortium for Top-Down Proteomics.

2014 International Representative of the French Society for Mass Spectrometry

2014 Member of the Royal Society of Chemistry (UK)

2012 Prime d'Excellence Scientifique

2011 High Potentials Leadership Training (CNRS), Promotion Marie Sklodowska Curie

Relevant publications, products or services

- Neisseria meningitidis type IV pili composed of sequence invariable pilins are masked by multisite glycosylation, J. Gault, M. Ferber, S. Machata, A-F. Imhaus, C. Malosse, A. Charles-Orszag, C. Millien, G. Bouvier, B. Bardiaux, G. Péhau-Arnaudet, K. Glinge, I. Podglajen, M-C. Ploy, H.S. Seifert, M. Nilges, J. Chamot-Rooke, G. Dumenil, *PLoS Pathog,* 11:e1005162 (2015).
- Conserved Streptococcus pneumoniae Spirosomes Suggest a Single Type of Transformation Pilus in Competence. R. Laurenceau, K. Petya, A. Diallo, S. Ouari, M. Duchateau, C. Malosse, J. Chamot-Rooke, R. Fronzes. *PLoS Pathog.* 11 (4) e1004835 (2015).
- Complete posttranslational modification mapping of pathogenic *Neisseria meningitidis* pilins requires top-down mass spectrometry. J. Gault, C. Malosse, S. Machata, C. Millien, I. Podglajen, MC. Ploy, C. E. Costello, G. Dumenil, J. Chamot-Rooke, *Invited Paper in Proteomics* DOI: 10.1002/pmic.201300394 (2014).
- **Post-translational Modification of Pili upon Cell Contact Triggers *N. meningitidis* Dissemination**. J. Chamot-Rooke, G. Mikaty, C. Malosse, M. Soyer, A. Dumont, J. Gault, A-F. Imhaus, P. Martin, M. Trellet, G. Clary, P. Chafey, L. Camoin, M. Nilges, X. Nassif, G. Duménil, *Science,* **331**, 778-782 (2011).
- Alternative Neisseria spp. type IV pilin glycosylation with a glyceramido acetamido 2,4,5-trideoxyhexose residue. J. Chamot-Rooke, B. Rousseau, F. Lanternier, G. Mikaty, E. Mairey, C. Malosse, G. Bouchoux, V. Pelicic, L. Camoin, X. Nassif, G. Dumenil, *Proc. Nat. Acad. Sci. USA, Proc. Nat. Acad. Sci. USA* 104, 14783-14788 (2007).

Relevant previous projects or activities

- *[2015-2018] Rapid identification of bacterial pathogens by top-down proteomics in a clinical context,* **French Research Agency (ANR)**, "**Health, Biomedical Innovation" challenge**, PI: J. Chamot-Rooke, Budget: 700 k€
- *[2015] Acquisition of a High Field, High Speed Mass Spectrometer (Q-Exactive HF) for the development of infectious disease proteomics,* **Institut Carnot Maladies Infectieuses,** PI: J. Chamot-Rooke, Budget: 200 k€
- *[2014-2016] Rapid identification of bacterial pathogens by top-down proteomics in a clinical context,* **Institut Pasteur PasteurInnov Call**, PI: J. Chamot-Rooke, Budget: 180 k€

Relevant Patents

"Recombinant aspartyl protease antigen or antibody thereto for use in immunotherapy of fungal diseases" Application Number EP15307114.7

Relevant infrastructure or equipment

Institut Pasteur has use of a standard mixture of 6 proteins from 8 to 66 kDa developed for intact protein analysis (with the Consortium for Top-Down Proteomics) Data has already been acquired for the analysis of this standard mixture with various LC-/MS/MS systems (Orbitrap Fusion Lumos and Orbitrap Q-Exactive)

Expertise in top-down analysis

Development of a E. coli standard sample for top-down proteomics

Expertise in sample preparation, top-down analysis and data acquisition for top-down proteomics

- Mass Spectrometers: 1 Orbitrap Fusion Lumos with ETD (TF), 1 Q-Exactive HF (TF), 2 Q-Exactive Plus (TF), 1 LTQ-Orbitrap Velos (TF), 1 Synapt G2SI HDMS with ion mobility, ETD (Waters) and HDX automated system (Leap Technology).
- All mass spectrometers are equipped with nanoLC systems
- Nanomate (Advion) for direct injection of samples
- Akta micro and HPLC for protein fractionation
- Analysis of complex bacterial samples obtained from various sources (IP collection, hospitals), in particular *Staphylococcus aureus* presenting different phenotypes of virulence or resistance

Main Tasks in TopSpec	**WP**
Institut Pasteur will test the different parts of the TopSpec system on standard protein mixtures, and system on real-life protein mixtures, and will apply the TopSpec MS/MS system to real-life analytical samples. Institut Pasteur will be active in the dissemination and communication activities in WP8.	2,3,4,5,7,8,9

4.1.8 Participant 8

Participant number	**8**
Organization full name	**MS Vision Technik und Wirtschaft – HTW Aalen)**
Organization Short name	MS
Website	http://msvision.eu/

Description of the Organisation
MS Vision is Europe's leading independent LC/MS service provider, offering support to hundreds of customers throughout Europe in a flexible and cost-effective manner. Established in 2004, we support with 12 field based engineers all current and old models of LC/MS platforms manufactured by Waters, Thermo and AB Sciex. MS Vision employs OEM trained senior engineers, working from their home office located in Germany, the UK and the Netherlands, supported by our logistics and repair facility in the Netherlands. We have a fully equipped laboratory for final test of complete LC/MS systems, these systems are also used to train our engineers on the current technology. MS vision also has a dedicated electronic workshop for repair and modification of electronic units used in Mass Spectrometry instruments. An important activity which has taken place continuously within MS Vision since 2005 is the modification of standard mass spectrometers for dedicated analysis of large proteins and protein complexes. In close cooperation with the group of Prof. Dr. Albert Heck at Utrecht University, hardware and software adaptations were developed and implemented for improved ion transmission and desolvation of high mass ions sprayed under native conditions. This has lead to the sales of ~30 modified mass spectrometers throughout Europe, which are currently maintained by MS Vision. Several development projects are now running at MS Vision and partners to further improve performance of these systems, in terms of selectivity (Ion Mobility project with Prof. Dr. Perdita Barran, Manchester University), sensitivity and desolvation (project with Fasmatech, Athens, Greece).
Key persons carrying out the research
Jan Commandeur (male) holds a BSc degree in Chemistry (Hogeschool Alkmaar, 1990). He continued his education at FOM Institute for Atomic and Molecular Physics (AMOLF) in Amsterdam, where he was involved in a wide variety of multi-disciplinary research projects as support technician. He joined Micromass (Waters) in 1998, and specialized in mass spectrometry support, both from a technical as a management point of view. In 2004 he was one of the co-founders of MS Vision, where he manages the service activities and is the driving force behind the various technology projects that lead to the sales of modified instrumentation for the analysis of large protein complexes, and that are currently underway (Ion Mobility, enhanced desolvation). **Silvio Keckes** (male) graduated at the Faculty of Physical Chemistry at the University of Belgrade in 2003. In 2011-2012 he continued with M.Sc. studies at the Department of Analytical Chemistry in Belgrade. From 2012 until today Silvio continues his education in the same faculty and prepares his PhD work. The main focus of his thesis is the multi-compound analysis of polyphenol compounds in food samples, pesticide residues in food samples, and method development/optimization on high resolution Mass Spectrometers for different analytes in food and environment samples. The major attention was drawn to the optimization of the LC/AMHR mass conditions with respect to the making of robust, fast, and sensitive methods. Silvio has attended a number of

service, application, and education training courses in different factories and training centres (Atlas Park Manchester; Waters Paris training centre; Thermo Scientific Bremen) where he focused on service, application and education in the different fields of Mass Spectrometry (Triple Quads, Ion Trap, OrbiTrap, Magnetic sector). He has co-authored on 5 scientific papers in peer-reviewed journals and contributed to a number of conferences with posters and oral presentation. Silvio worked as product manager for chromatography and Mass Spectrometry for Analysis d.o.o for Serbia, Bosnia and Herzegovina, Montenegro, Macedonia, and Albania providing sales, marketing, service, application and education for related products. At MS Vision he works as technical support specialist for Thermo MS equipment with a strong focus on Orbitrap technology.

Relevant publications, products or services

Relevant products are engineering skills both in installation as well as maintenance of high end MS equipment and two research engineers, each with more than 20-year experience in R & D in the field of Mass spectrometry. MS Visions track record in development and manufacturing of major enhancements of TOF technology for High Mass analysis such as Native MS is recognised and proven.

Relevant previous projects or activities

Dutch STW project (no 10805), Development of analytical tools for the molecular and structural characterization of therapeutic monoclonal antibodies and mixtures of monoclonal antibodies and for the analysis of their interaction with their therapeutic targets.

MS Vision employs two research engineers, they work with the group of Prof. A. Heck, University of Utrecht, NL on improvements of Waters Q-Tof and Synapt based systems for Native MS applications. Currently more than 30 systems have been modified and sold in Europe to leading groups in academia and Pharmaceutical companies.

Another project our research team works on is on Ion Mobility, again on TOF technology in collaboration with the group of Prof. P. Barran, Manchester University UK (EPSRC IAA Exploitation Secondment project "Integrating Drift Tube Ion Mobility to existing Mass Spectrometers".

Relevant infrastructure or equipment *(in relation to the present proposal)*

Our own fully equipped laboratory, with additional wet lab for sample preparation and storage, at our facility in the Netherlands is ideal for assembly and test of LC/MS systems. Together with our electronic workshop, this allows us to work on systems under development and test them in great detail.

Main Tasks in TopSpec	**WP**
MS Vision will be responsible for WP8, participate in TopSpec testing and investigate the market for technology, including preparation of the Business plan in collaboration with all partners.	5,8,9

4.2.

No subcontracting and/or third parties are foreseen.

Section 5: Ethics and Security

5.1 Ethics

The procedures and criteria that will be used to identify/recruit research participants:

No human will be involved in the project, therefore there will be no need to identify or recruit participants.

The informed consent procedures that will be implemented for the participation of humans:

No human will be involved in the project; therefore there will be no need for such procedures.

The applicant must clarify whether children and/or adults unable to give informed consent will be involved and, if so, justification for their participation.

No human will be involved in the project; therefore there this is not an issue.

Details on incidental findings policy

Incidental findings from the biobanked samples will be presented and discussed with the clinician for further validation. If applicable the patient will be informed if agreed upon.

Human cells/tissues:

Biobanked samples from available serum enriched human IgG from projects (with clinical permits for protein/IgG analysis) will be used to test disease and/or phenotype characterization(s). We have access to polyclonal IgG from 1) patients with early Alzheimer's disease and patients with Lewy body dementia, study approved by the Norwegian regional Ethics Review Board approval number 2010/633, 2) patients with mild Cognitive Impairment, study approved by the Regional Ethical Review Board in Stockholm, Sweden, 3) Sepsis patients, study approved the Regional Ethical Review Board in Örebro, Sweden, EPN Dnr. 2014/193, 4) sarcoidosis patients, study approved by the regional ethical review board Stockholm, Sweden, 2005/1031–31/2.

Details on the materials which will be imported to/exported from the EU:

No material will be imported to/exported from the EU. (The IgG samples from the Norwegian cohort are already stored at Karolinska Institutet, Stockholm, Sweden).

If human subjects are indeed involved in the experimentation tasks of the project, copies of relevant opinions/approvals by ethics committees must be kept on file and submitted to the REA upon request.

No human will be involved in the project; therefore there this is not an issue.

Data privacy

The project does not require the collection and processing of personal data in the form of medical records. All clinical information relevant for disease/phenotype characterization is obtained through clinicians.

5.2 Security

Based on article 37.1 of the Model Grant Agreement, there are no activities or results which will be raising security issues related to this proposal.