EUROPEAN COMMISSION
EUROPEAN INNOVATION COUNCIL AND SMES EXECUTIVE AGENCY

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GENERAL PROJECT REVIEW CONSOLIDATED REPORT

Grant agreement (GA) number:	829157
Project[1] Acronym:	TopSpec
Project title:	Next generation precision antibody profiling - from science fiction to reality
Type of action:	RIA
Start date of the project:	01/01/2019
Duration of the project:	42
Name of primary coordinator contact and organisation:	Roman ZUBAREV (KI)
Period covered by the report:	from 01/01/2019 to 03/09/2021
Periodic report/Reporting period number:	Assessment not linked to the end of a reporting period
Date of first submission of the periodic report (if applicable):	Not applicable
Amendments (latest AMD concerning description of the action)[2]	19/11/2020 (AMD-829157-12)
Date of meeting with consortium (if applicable):	22/07/2021
Name of project officer:	Ioannis FIAMEGKOS
Name(s) of monitors:	– Juan Antonio VIZCAINO GONZALEZ • EMBL-European Bioinformatics Institute • EMBL-European Bioinformatics Institute • EMBL-European Bioinformatics Institute – Christophe Dominique Masselon • CEA • Pacific Northwest National Laboratory – Stefanie Monika HAUCK • Helmholtz Zentrum München - Research Unit Protein Science • Helmholtz Zentrum München • Helmholtz Zentrum München

[1] 'Project' means the same thing as 'action'.
[2] Only amendments to the description of the action (DoA; AT21) are relevant for general project reviews since they always have to be carried out against the latest version of the DoA

1. Overall assessment

1. Overall assessment

Project has achieved most of its objectives and milestones for the period with relatively minor deviations.

2. Significant results linked to dissemination, exploitation and impact potential

Project has delivered exceptional results with significant immediate or potential impact (even if not all objectives mentioned in the Annex 1 to the GA were achieved).

During the reported period, and in spite of the COVID-19 pandemic, the TOPSPEC consortium has made tremendous progress toward their stated goals of characterizing intact antibodies by top-down mass spectrometry. The results reported are very significant, especially at the level of the development of the Omnitrap platform, but also in software development. This is quite remarkable, given the instrumentation and analytical challenges this represents and the highly multinational and integrated nature of the project.
Overall, the project has achieved most of its deliverables (7 out of 9; two deliverables are delayed but likely will be delivered as expected) and all the milestones for the period (M6 and M7).

During the second review meeting, the consortium showed excellent results related to the proposed workplan and even went beyond the original proposal. More concretely, the consortium did excellently solve numerous anticipated and non-anticipated obstacles. The following ones could be highlighted, split by WP:
- WP1: Partner FASMATECH developed the Omnitrap platform and connected it to the Orbitrap mass spectrometers to be then delivered to France and Sweden (D1.1). Constructions are nearly finished, although the delivery to receiving labs has been delayed and it is still pending. Additionally, first ever MS4 analysis of intact antibodies was achieved on the Omnitrap QE platform.
- WP2: Implementation of activation techniques (slow heating CID, ECD, ….) was successful.
- WP3: Hybrid hyperthermal-atom ion source for activation-dissociation was developed and patented.
- WP4: Developments related to Coulombic explosion dissociation via high energy electron impact ionization: a prototype CE gun was installed and tested. Optimization is currently ongoing.
- WP5: Development of pI-Trap-ESI combination: pI –Trap with autosampler was built and delivered and the controlling software was also delivered. Additionally, experiments with mAb separated in the pI-Trap were performed.
- WP6: Modification of the Orbitrap mass spectrometer: An Exploris 480 instrument has been delivered to partner FASMATECH. The modification of the instrument for an Omnitrap connection was achieved and the related software developments efforts were delivered as well. The initial experiments showed >90% efficiency.
- WP7: Developments related to signal detection (two prototypes developed and installed) and data processing (several manuscripts submitted) were achieved.

3. General comments

The TOPSPEC Consortium has done a very good job overall, especially considering all the extra difficulties that have arisen due to the COVID-19 pandemic. There have been inevitable delays, but it is clear that the Consortium has managed to accomplish a lot during this reporting period. Overall, the consortium has coped with this difficult situation in an excellent manner, the delay appears rather minor and can be mitigated within the extension that has been granted.

It should be highlighted that partner FASMATECH in interactions with THERMO FISHER SCIENTIFIC and the KAROLINSKA INSTITUTE has shown great progress in spite of significant technical challenges. They went above and beyond the initial prospects and had to rely on internal resources to do so. Partners involved in the data processing (NTU) and analytical aspects (IP) linked with Antibody characterization also showed excellent progress. The contributions from the SMEs in charge of the novel detector and the antibody separation respectively were more incremental over the period, but they seem poised for the final stage of the project. Two patents have been filed and several articles have either been submitted or are on their way. The consortium is very active and making coordinated progress.

4. Recommendations concerning the period covered by the report

No major deviation from workplan is reported. However, some improvements in the technical report are still possible. These recommendations should be considered for future technical reports:
- From the technical report alone, it is sometimes hard to understand the integration and relationship between the different tasks and deliverables, both for hardware and software.
- Inclusion of specific sections at the end of each WP would be helpful to better appreciate the integration between the different tasks and deliverables achieved by the different partners. A summary of activities in WP1-WP4 is already included in the report, but this represents just a first step.

- Inclusion of a figure where all software tools included in the report are included (developed by partners FASMATECH, UNIV. NOTTHINGHAM and INSTITUT PASTEUR), indicating where they do fit in the overall top-down proteomics data acquisition and data analysis workflow.

Additionally, the review meeting should have included a section devoted to Dissemination and outreach activities.

5. Recommendations concerning future work, if applicable

The following recommendations are suggested:
- The consortium members need to decide as soon as possible on a plan for the future exploitation and sustainability of the developed software during the project (relevant for WP1 and WP7). Different options are available, ranging from completely open-source software to fully paid licensed software, with the aim of financing the provision of service to the software users. All options are possible and have advantages and disadvantages. A clear plan needs to be decided.
- A major requirement for natural antibodies analysis is their pre-separation, and the proposed IEF fractionation seemed a very interesting prospect. IEF offers the best possible peak capacity and defocusing by diffusion should be quite minimal for molecules as large as antibodies. However, several technical as well as organizational hurdles seem to have jeopardized this effort. While progress has been shown, it still seems far from delivering the performance required for efficient antibody separation. The concerned parties may want to refer to earlier work from the R.D. Smith group on capillary IEF of intact proteins and protein complexes for ways to improve their setup (see in particular the detailed protocol published in MiMB: Martinović S, Pasa-Tolić L, Smith RD. Capillary isoelectric focusing-mass spectrometry of proteins and protein complexes. Methods Mol Biol. 2004;276:291-304. doi: 10.1385/1-59259-798-X:291) (see also: Jensen PK et al. Mass spectrometric detection for capillary isoelectric focusing separations of complex protein mixtures. Electrophoresis. 2000 Apr;21(7):1372-80. doi:10.1002/(SICI)1522 2683(20000401)21:7<1372::AID-ELPS1372>3.0.CO;2-Y.).
- Dissemination and outreach activities need to be improved. Of course, the impact of the COVID-19 pandemic has made face-to-face events not feasible, but has also open many possibilities for virtual and hybrid events. A section reporting these activities should be included in the next review meeting.

2. Objectives and workplan

1. Is the progress reported in line with objectives and work plan as specified in the DoA? If there are significant deviations, please comment.	Partially
The reported progress matches the objectives specified in the DoA. All objectives have been followed accordingly and small adaptations have been made only to overcome technical issues and hurdles. More concretely, technical challenges related to the Omnitrap instrumentation entailed additional work, which has been performed using internal resources (partner FASMATECH). Minor deviations from the original workplan in terms of timing directly resulted from the pandemic situation and the resulting International travel limitations.	
2. Are the objectives of the project still scientifically and /or technologically relevant?	Yes
The objectives remain highly relevant and the overall goal of TOPSPEC is probably more important than ever now, since the ongoing COVID19 pandemic puts therapeutic antibodies even more at a centre stage for human health issues. The possibility to achieve complete sequence coverage of an intact antibody, which has been demonstrated within this reporting period, has the prospect to advance antibody characterization for applications in biology and pharmacology.	
3. Are the critical implementation risks and mitigation actions described in the DoA still relevant?	Yes
The critical implementation risks and mitigation described in the DoA are still relevant. As should be expected, some of the anticipated risks did not materialize, while unanticipated technological issues emerged. Overall, the consortium has managed to stay on course, and has shown remarkable progress toward most of their objectives. The risk highlighted during the previous reporting period (the coupling of the Omnitrap with the Exploris spectrometer) has been successfully mitigated. The problems related with the outgasing of the PEEK material have also been successfully tackled by performing a complete re-designing of the Omnitrap with using minimal PEEK material. The risk imposed by the strayed magnetic field on deflecting the ion beam, that has to be consolidated with every hardware change, should be clearly identified in the final report.	
4. Have the ethics deliverables due for the current period been adequately addressed and approved?	Not applicable
There are no ethical concerns in this project.	
5. Have the comments and recommendations from previous project reviews been taken into account?	Yes
Previous recommendations pertaining to the complexity of the parameter space of multistage MSn experiments were taken into account, in particular by implementing the so-called 'bundles' in the instrument control software, allowing a faster design of experiments using pre-defined sets of parameters. Additionally, the recommendation to couple the Omnitrap to the Exploris instrument has been successfully implemented.	

3. Impact

1. Is there any potential that the work carried out within the project will initiate or consolidate a new line of technology and its future uses? Can the project claim to have a transformational impact on technology and/or society?	Yes
The developments of the TOPSPEC consortium will have transformative impact on the analysis of intact antibodies (the work carried out will provide a novel analytical tool to characterize antibodies with unprecedented speed and sensitivity), and also on top-down proteomics in general. Top-down proteomics approaches have a lot of potential, not only for sequencing directly antibodies, but also for many other biological applications, where the study and characterisation of particular proteoforms will be key. Additionally, many of the technical developments in mass spectrometry instrumentation and software are of broader interest for other proteomics approaches.	
2. Do the interdisciplinary collaborations within the project bridge symbiotically advanced scientific and technological fields? Please, comment if the interdisciplinary collaborations result in the proposition of novel research and innovation practices.	Yes
The TOPSPEC team is per se highly interdisciplinary, integrating excellence in engineering, physics, chemistry, analytics, biochemistry, software development and data science. Their collaboration is expanding the potential of MS for the characterization of intact antibodies, a key requirement to boost our understanding of immune responses and towards the development of tailored immuno-therapies for e.g. cancer treatment. Integrations towards the analysis of other intact large proteins will have an even greater impact.	
3. Does the work carried out contribute towards European policy objectives and strategies and have an impact on policy making?	Yes
The TOPSPEC project contributes to at least three of the four pillars of the EU H2020 policy objectives and strategies: - Building ecosystems through mutual complementation among industries: five of the eight partners involved are from industry. - Solving social problems through science and technology: TOPSPEC will benefit the healthcare for the EU population, by helping e.g. in the development of immunotherapies to the benefit of cancer patients. - Strengthening SMEs' participation: four out of eight partners are SME, thus TOPSPEC has a large economical impact, by creating new jobs in existing SME and contributes to educating highly qualified personnel.	
4. Does the project involve new and high potential actors? Do they contribute to strengthen European scientific and industrial leadership in the early exploration of new and emerging technologies? Does the contribution go beyond academic excellence and with global recognition? (New potential actors = young excellent researchers, high-tech innovative SMEs).	Yes
TopSpec involves 4 SMEs: partners FASMATECH, SPECTROSWISS, BIOMOTIF and MSVISION. Especially the dedicated physics and technical engineering team at FASMATECH (founded in 2009) is composed of very promising high potential actors. FASMATECH as well as SPECTROSWISS (founded in 2014) and BIOMOTIF are high tech innovative SMEs. All these companies have shown remarkable contributions toward solving some of the technological and scientific challenges the consortium is facing, with FASMATECH taking the lead in the engineering. Therefore, these companies and in particular FASMATECH, have gone beyond the proposed work to deliver instrumentation and software that will undoubtedly change the way antibodies are characterized. The contributions will clearly go beyond academic recognition and put these SMEs in the position to take leadership in emerging technologies. Global recognition will be enabled by the connection to THERMO FISHER SCIENTIFIC, which is a global leader in mass spectrometry technology. TOPSPEC also involves young researchers of excellence and the project will help them in their international recognition.	
5. Have the beneficiaries reached gender balance at all levels of personnel assigned to the action? If not, have the reasons been explained in the periodic report?	No
The gender balance of the consortium as stated on the participant portal is 25% Female / 75% Male, so gender balance has not been achieved. While this can partly be explained by the imbalance in the fields of physics and instrumentation, this topic was not been discussed in the periodic report or during the review meeting.	

4. Implementation

1. Has the project been efficiently and effectively managed?	Yes
The high quality of the TOPSPEC achieved results, the innovative implementation of diverse technologies as well as the excellent handling of technical hurdles as outlined in the report and during the review meeting (including excellent scientific presentations) testifies the efficient and effective management of the project.	
2. Is the management of the project in line with the obligations of beneficiaries (including ethics and security requirements, risk and innovation management if applicable)?	Yes
There seems to be no obvious deviations from the obligations of the beneficiaries. Ethics and security requirements are not relevant in this project.	
3. Is the contribution of each beneficiary in line with the work committed in the DoA? (applicable only to multibeneficiary projects)	Partially
The TOPSPEC partners have all contributed as planned to the DoA and brought forth evidence of their commitment to the overall project goal. It should be highlighted that partner FASMATECH has put double the effort as originally planned to overcome the technical hurdles arising during the project. Additionally, as highlighted in the general recommendations regarding future work, the work related to the separation of antibodies using IEF fractionation (led by partner BIOMOTIF) is a bit delayed due to both technical and organisational hurdles.	
4. Have the beneficiaries disseminated project results (foreground) in scientific publications as planned in the DoA (including the deposition of publications in open access repositories)? Do they include a reference to EU funding?	Yes
The consortium has published four research articles in peer-reviewed journals so far (as listed on the TOPSPEC website) and properly credit funding from EU in all of them. All of them are open-access publications. Several other articles are at various stages of the publication process. 2 patents have also been filed (one from BIOMOTIF, one from FASMATECH).	
5. Have the beneficiaries disseminated and communicated project activities and results by other means than scientific publications (social media, press-release, the project web site, video/film, etc) as planned in the DoA? Do they include a reference to EU funding?	Yes
TOPSPEC beneficiaries have made communications at various conferences before the onset of the pandemic, and since then the focus has been put in virtual conferences. The project website appears updated and references the EU funding (https://topspec.ki.se/). Twitter and LinkedIn accounts are also active.	
6. Has the plan for the exploitation and dissemination of the results (if required) been updated and implemented as described in the DoA, in particular as regards intellectual property rights? Is it appropriate?	Yes
An IP protection strategy (D8.1) and a draft exploitation plan and business strategy (D8.2) have been created, reviewed and submitted. They are quite general and appropriate for the scope of the project. It would be adequate to update them at this stage, given the large amount of patentable innovation created in this project and the potential follow-up.	
7. Has the data management plan (DMP) (if required) been updated and implemented? Is it appropriate?	Partially
A Data Management Plan (D9.2) has been established and was submitted in June 2019. It appropriately describes the procedures and methods used by the consortium to store, manage and, if applicable, disclose all data pertaining to the project. The reviewers could not assess whether it was properly implemented or not. This topic was not mentioned in the review meeting.	

5. Resources

1. Were the resources used as described in the DoA and were they necessary to achieve its objectives? If there are deviations from planned budget, have they been satisfactorily explained? Have they been used in a manner consistent with the principle of sound financial management (in particular economy, efficiency and effectiveness)?	Not applicable
This was an intermediate review meeting and only the technical/scientific part was evaluated. The relevant financial information and the 'Use of Resources' was not fully provided.	

Annex 1

Expert opinion on deliverables

Deliverable number	Deliverable name	Status	Comments
D1.3	Fully serviced, functioning Omnitraps & IMS	Accepted	
D2.1	in situ testing of the optimized CAD MS/MS protocol	Accepted	
D2.2	Protocol of in situ testing of the optimized CAD	Accepted	
D2.3	in situ testing optimized ECD, HECD and EID MS/MS	Accepted	
D3.1	Prototype of the HAB gun installed and tested protocol	Accepted	
D3.2	Optimized HAB guns installed and tested - protocols	Accepted	
D4.1	Protocol- CED gun prototype installed and tested	Accepted	
D5.1	Prototype pI-Trap-ESI installed and tested– protocol	Accepted	
D9.4	Scientific review meeting documents	Accepted	

Annex 2

Expert opinion on milestones

Milestone number	Milestone name	Achieved	Comments
MS1	Demonstrated effectiveness of product ion isotopic distribution deconvolution	Yes	Done
MS2	Omnitraps & IMS Electronics design	Yes	Achieved quite some time ago
MS3	Installation of Q Exactive instrument for Omnitrap development	Yes	Achieved quite some time ago
MS4	Omnitraps & IMS P.O.s sent to suppliers	Yes	milestone has been achieved
MS5	Omnitraps & IMS Mechanical design	Yes	milestone has been achieved
MS6	Data processing algorithms and software for simulated and experimental top-down mass spectra and time-domain data transients	Yes	milestone has been achieved
MS7	Suffic HAB MS/MS demonstrated	Yes	This has been achieved