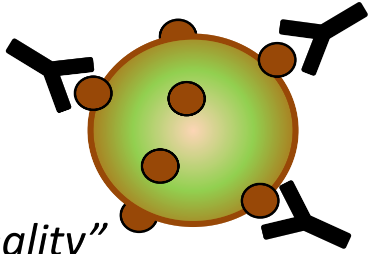
9.45 - 10.15 Overview of the project and its general status

Background

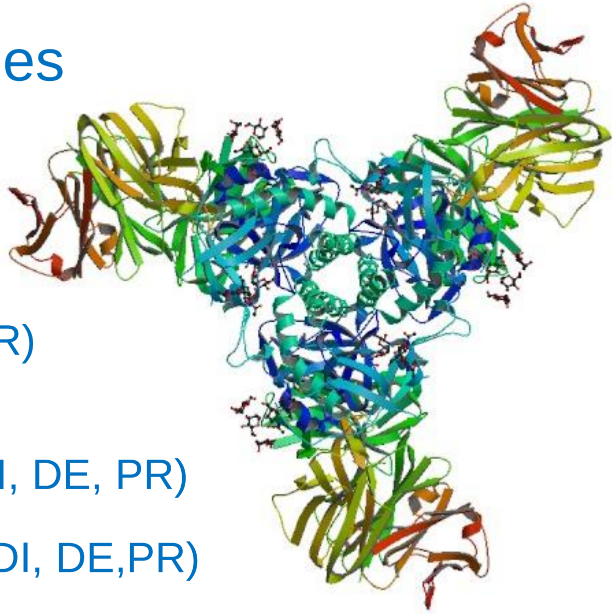


“Next generation precision antibody profiling – from science fiction to reality”

- **Vision:** diseases are cured by body’s own immune system.
- **Challenge:** cost of developing targeted immunotherapies.
- **Ultimate aim**: Reducing time/cost of novel immunotherapies.
- **Obstacle**: Understanding how antibodies are produced in humans.
- **Antibodies:** macro-proteins (130-1000 kDa) binding to targets (antigens) via variable sequences. Astronomical number of sequences exist theoretically, but tiny fraction of all possible sequences are actually generated and selected.
- **Specific aim**: sequencing antibody repertoire and correlating it with disease/antigen to understand the rules of production/selection.

TopSpec’s solution to this challenge: top-down mass spectrometry sequencing platform

Characterization of Antibodies



Why Antibodies ?

Immunotherapy

Biotherapeutics & Biosimilars
(mAbs, ADC)

Medical Diagnostics (ELISA)

Life Sciences R&D

Why Top-Down ?

Degradation products – (DE,PR)

Proteoform Characterization
(SS scrambling, Glycans) – (DI, DE, PR)

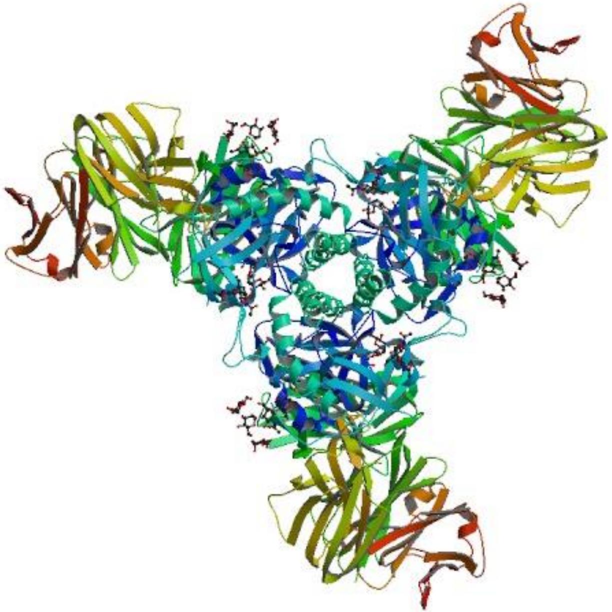
Structural Characterization – (DI, DE,PR)

mAb Design & *De Novo* sequencing – (DI)

Pharma - Biologics

DIscovery → DEvelopment → PRoduction → MArket

Sequencing of Antibodies



State-of-the-art Limitations

Bottom-Up Protein ID & *de novo* sequencing

Sub-optimal for determining modification occupancy

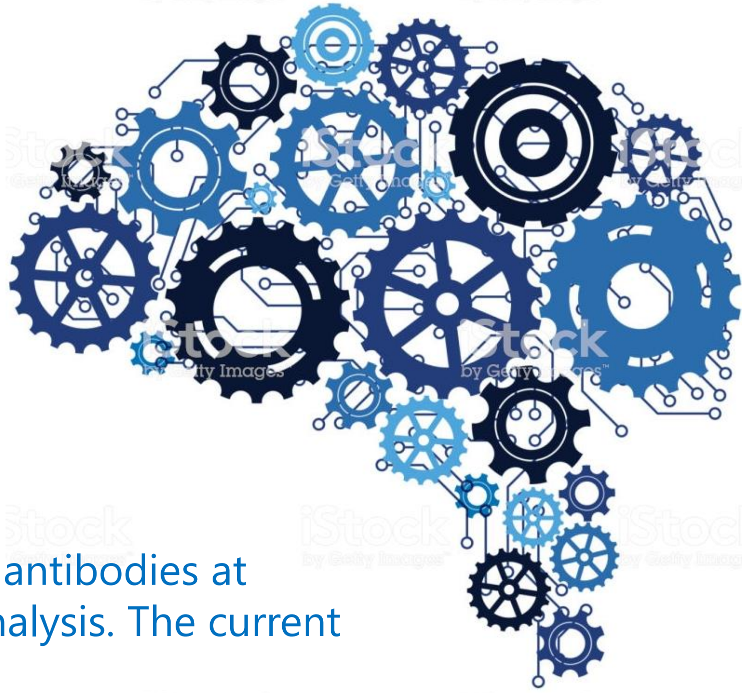
Proteoforms are not accessible

Speed & Accuracy

Identification & sequencing of multiple proteins (mAbs) with unknown sequence simultaneously from a complex sample is not possible in bottom-up.

Top-Down *de-novo* sequencing of proteins from complex samples is feasible

TopSpec Platform



We are enabling the future of antibody characterization

We envision:

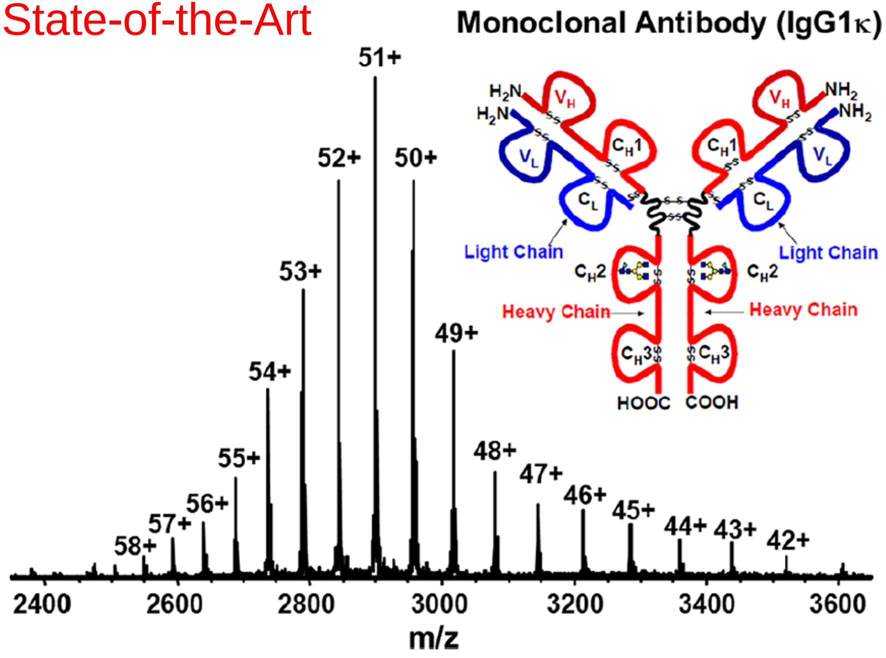
- **Analysis of complex samples** – *de novo* sequencing of antibodies at proteoform level in complex samples with top-down analysis. The current state-of-the-art is limited to 3-5 mAbs in a mixture
- **Improvements in reliability/speed/cost** – Today *de novo* sequencing is time consuming and expensive - reducing analysis time and cost is a major step forward

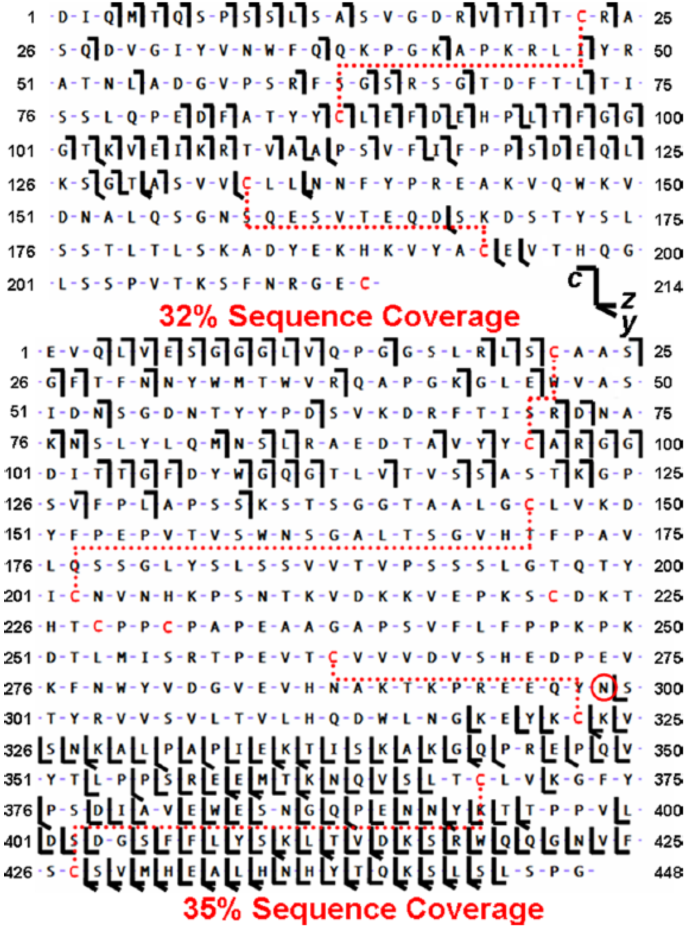
TopSpec Innovations

- Novel hardware to isolate antibodies and analyze their ions in the gas phase
- Novel ion activation techniques & methods in tandem MS
- Novel signal processing in Fourier transform MS
- Novel data analysis algorithms for top-down sequencing

Top Down Sequencing of Antibodies

State-of-the-Art





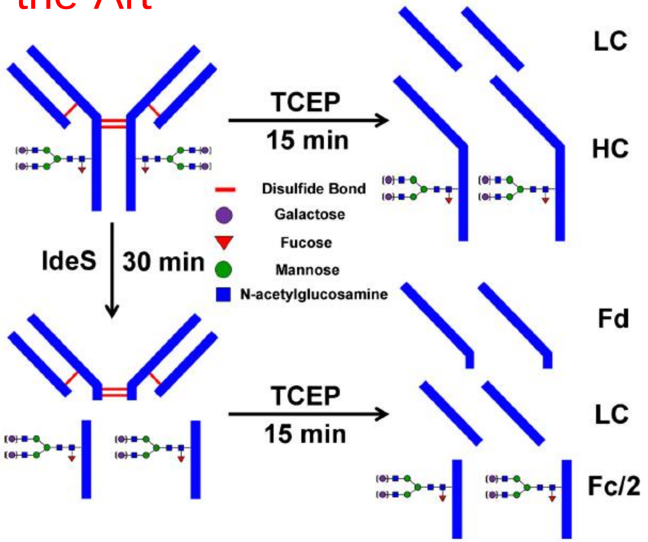
Fragments are mainly localized:

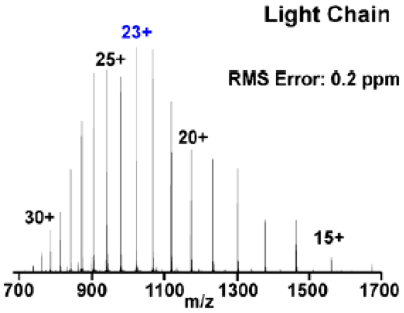
a. in the variable region of light and heavy chains
b. between variable and constant domains in both chains
c. between heavy chain constant domains C_H2 and C_H3
d. In the S-S linked heavy chain constant domain C_H3

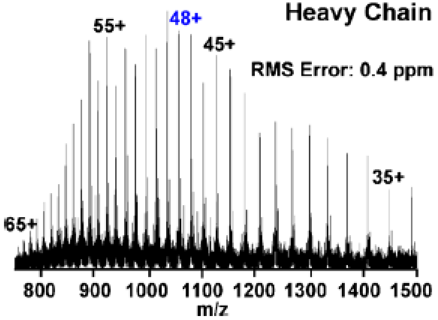
Marshall A *et al*, Anal Chem 2013, 85, 4239

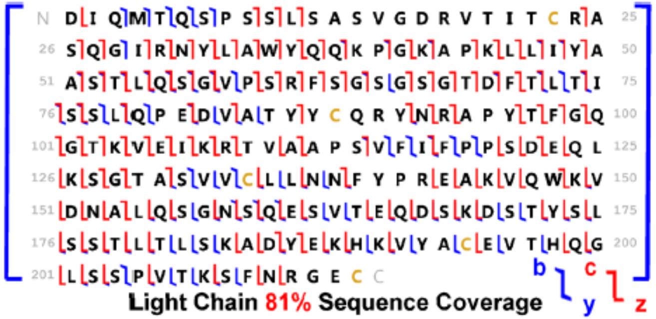
Middle Down Sequencing of Antibodies

State-of-the-Art

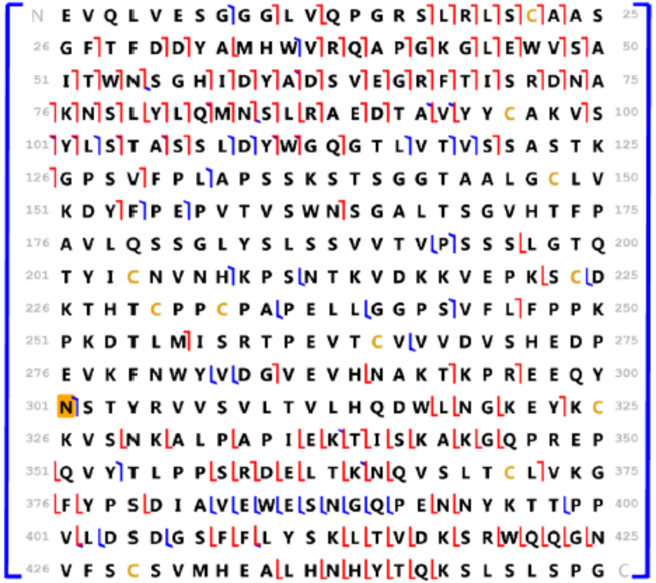








Light Chain 81% Sequence Coverage

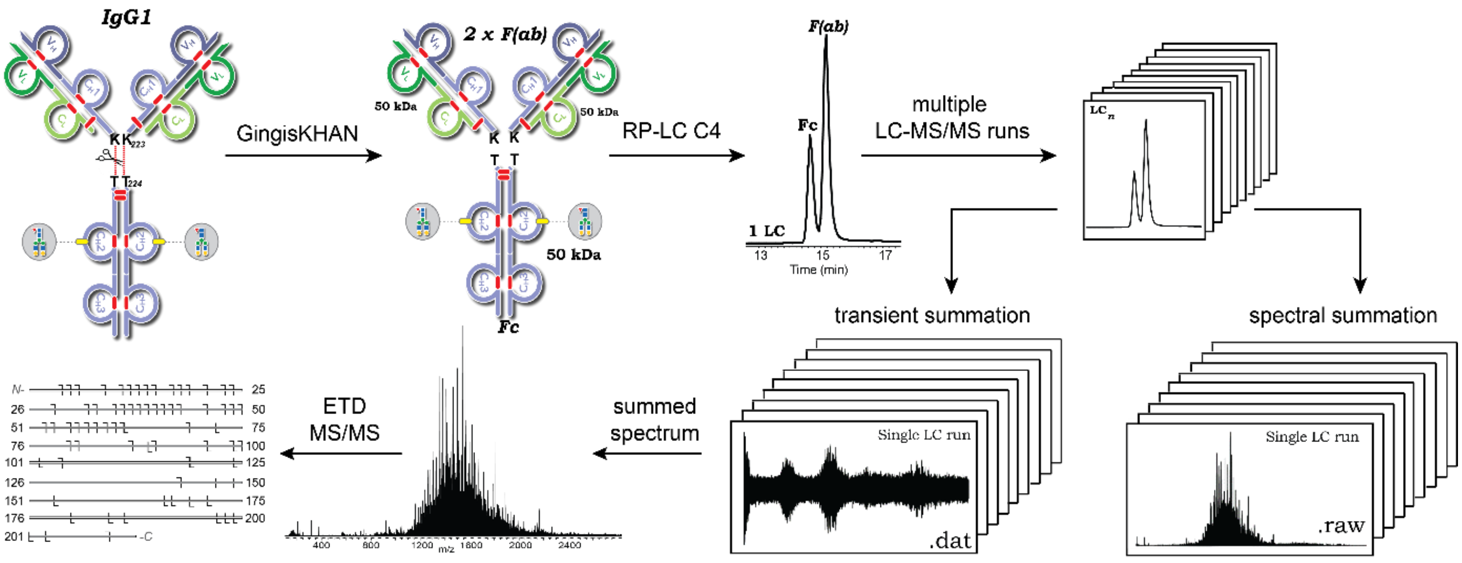


Heavy Chain 38% Sequence Coverage

Marshall A *et al*, J Am Soc Mass Spec, 2017, 28, 827

Middle Down Sequencing of mAbs: Light and Heavy Chain Pairing (50 kDa)

State-of-the-Art



GingisKHAN

RP-LC C4

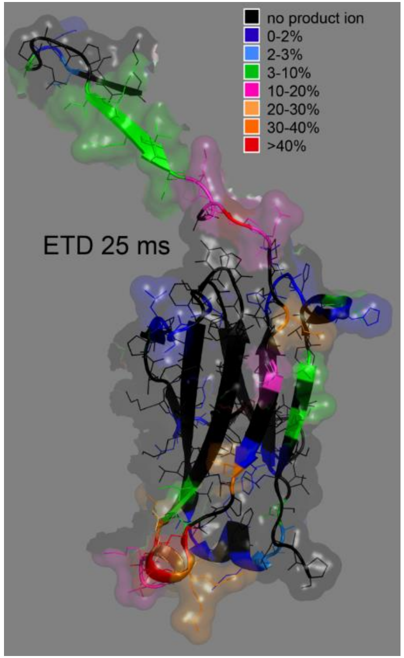
multiple LC-MS/MS runs

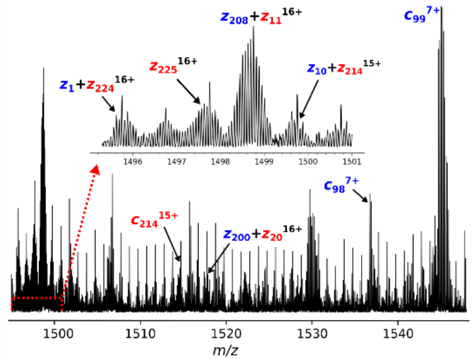
transient summation

spectral summation

summed spectrum

ETD MS/MS

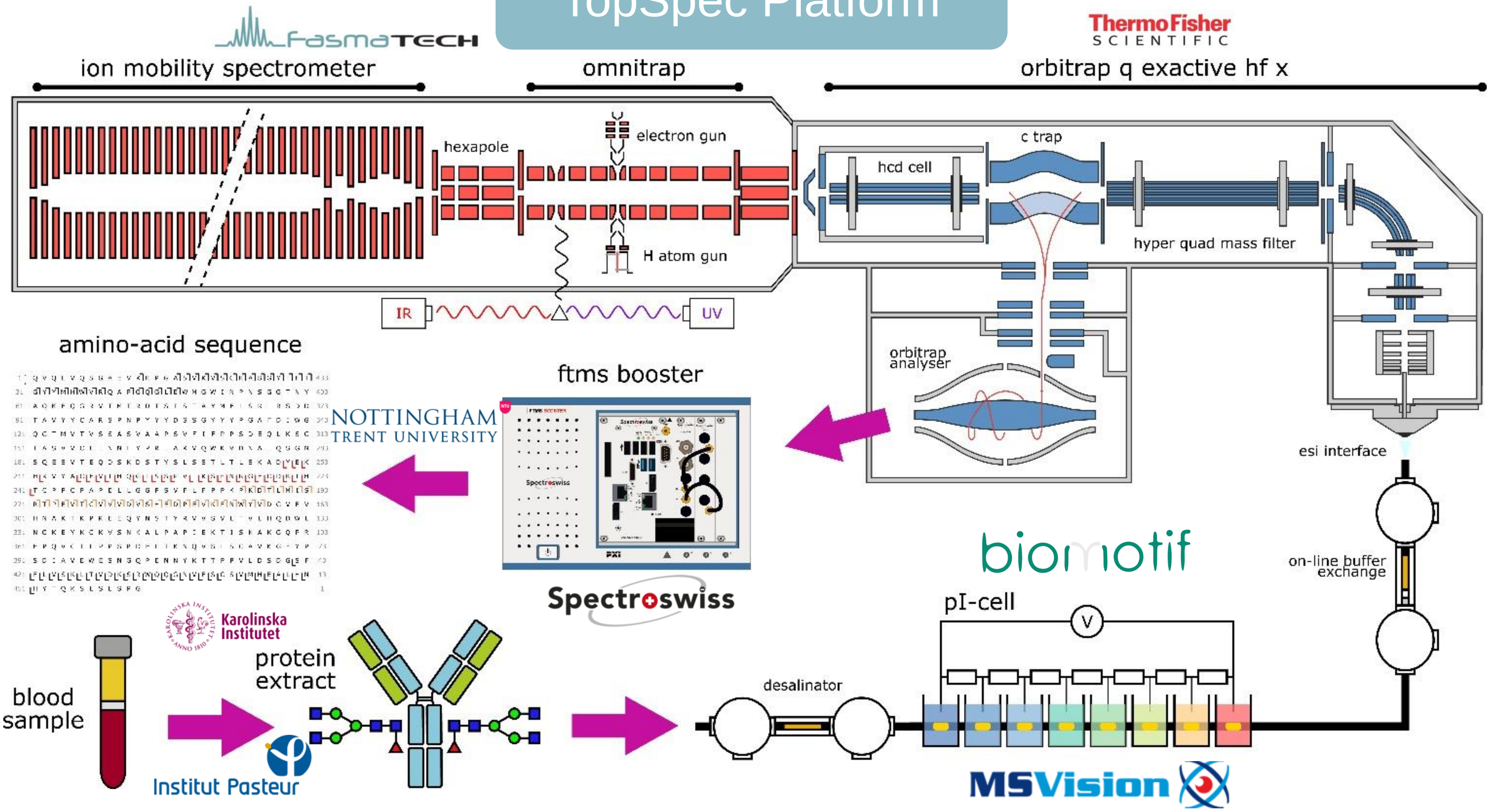




Data processing approach	LC runs	Sequence coverage, %	
		Light Chain	Heavy Chain
Transient	10	**34.0**	**41.9**

Tsybin *et al*, Anal Chem, (2018) 12527

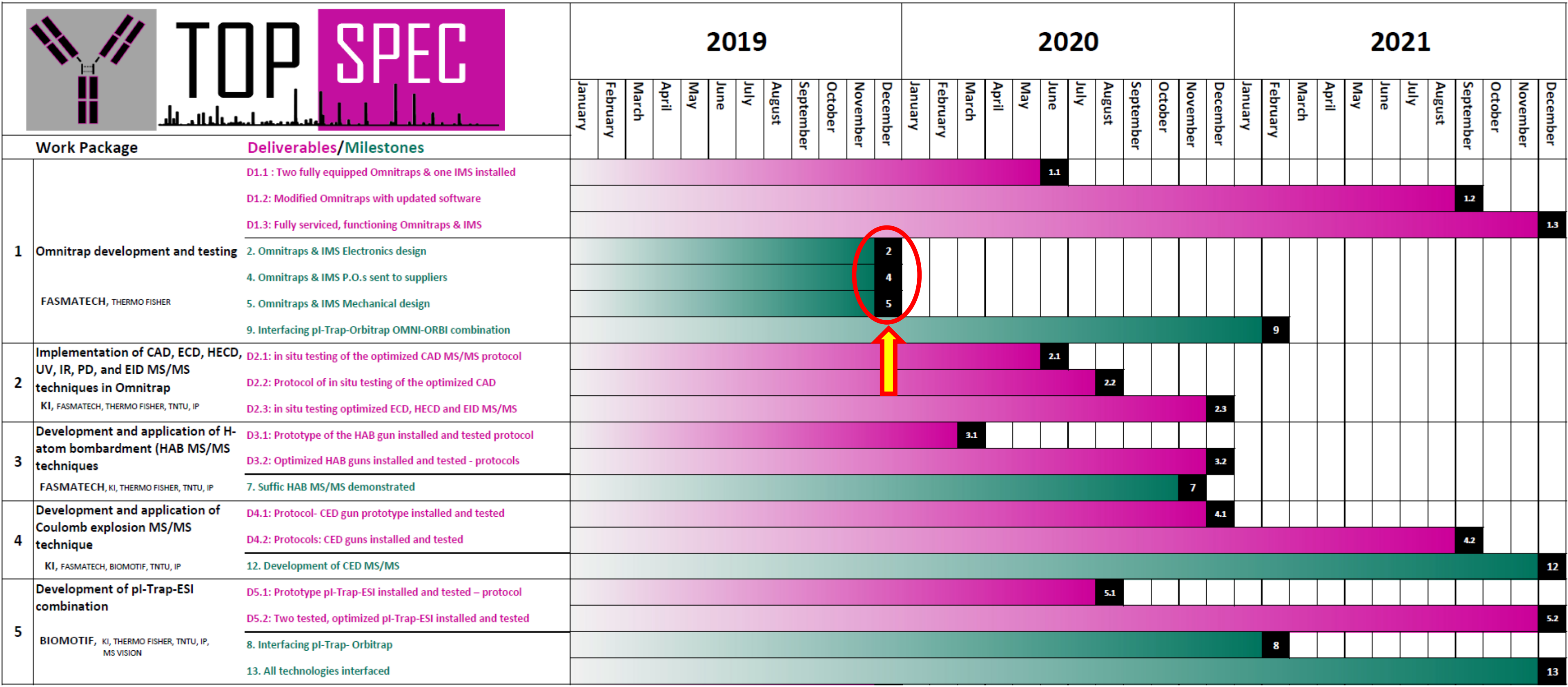
TopSpec Platform



TopSpec Work Packages

WP**1**	Omnitrap development and testing	→	**FT, TF: 3 deliverables**
WP**2**	Implementation of CAD, ECD, HECD, UV, IR, PD, and EID MS/MS techniques in Omnitrap	→	**KI, FT, TF, TNTU, IP: 3 deliverables**
WP**3**	Development and application of H-atom bombardment (HAB MS/MS techniques)	→	**FT, KI, TF, TNTU, IP: 2 deliverables**
WP**4**	Development and application of Coulomb explosion MS/MS technique	→	**KI, FT, B, TNTU, IP: 2 deliverables**
WP**5**	Development of pI-Trap-ESI combination	→	**B, KI, TF, TNTU, IP, MV: 2 deliverables**
WP**6**	Modification of the Orbitrap mass spectrometer	→	**TF, KI: 2 deliverables**
WP**7**	Signal detection and data processing	→	**S, KI, FT, TNTU, IP: 3 deliverables**
WP**8**	Dissemination, Communication & Exploitation	→	**MV, KI, FT, TF, S, B, TNTU, IP: 8 deliverables**
WP**9**	Project Management and Administration	→	**KI, FT, TF, S, B, TNTU, IP MV: 4 deliverables**

TopSpec Deliverables & Milestones



			2019												2020												2021											
	Work Package	Deliverables/Milestones	January	February	March	April	May	June	July	August	September	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December
1	Omnitrap development and testing FASMATECH, THERMO FISHER	D1.1 : Two fully equipped Omnitraps & one IMS installed																		1.1																		
		D1.2: Modified Omnitraps with updated software																																1.2			
		D1.3: Fully serviced, functioning Omnitraps & IMS																																			1.3
		2. Omnitraps & IMS Electronics design												2																							
		4. Omnitraps & IMS P.O.s sent to suppliers												4																							
		5. Omnitraps & IMS Mechanical design												5																							
		9. Interfacing pI-Trap-Orbitrap OMNI-ORBI combination																										9										
2	Implementation of CAD, ECD, HECD, UV, IR, PD, and EID MS/MS techniques in Omnitrap KI, FASMATECH, THERMO FISHER, TNTU, IP	D2.1: in situ testing of the optimized CAD MS/MS protocol																		2.1																		
		D2.2: Protocol of in situ testing of the optimized CAD																				2.2																
		D2.3: in situ testing optimized ECD, HECD and EID MS/MS																								2.3												
3	Development and application of H-atom bombardment (HAB MS/MS techniques FASMATECH, KI, THERMO FISHER, TNTU, IP	D3.1: Prototype of the HAB gun installed and tested protocol															3.1																				
		D3.2: Optimized HAB guns installed and tested - protocols																								3.2												
		7. Suffic HAB MS/MS demonstrated																							7													
4	Development and application of Coulomb explosion MS/MS technique KI, FASMATECH, BIOMOTIF, TNTU, IP	D4.1: Protocol- CED gun prototype installed and tested																								4.1												
		D4.2: Protocols: CED guns installed and tested																																4.2			
		12. Development of CED MS/MS																																			12
5	Development of pI-Trap-ESI combination BIOMOTIF, KI, THERMO FISHER, TNTU, IP, MS VISION	D5.1: Prototype pI-Trap-ESI installed and tested – protocol																				5.1																
		D5.2: Two tested, optimized pI-Trap-ESI installed and tested																																			5.2
		8. Interfacing pI-Trap- Orbitrap																										8										
		13. All technologies interfaced																																			13

Milestones:T2/T5. Omnitraps & IMS Electronics design (FT, Dec 31, 2019)

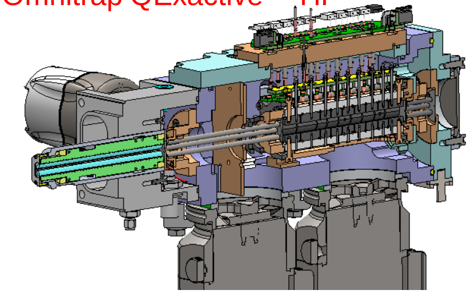
Precursor Omnitrap

- Designed ✓
- Installed ✓
- Tested ✓
- Revisions ✓

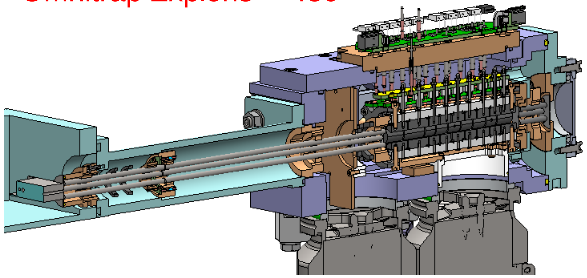
Precursor IM drift cell

- Designed ✓
- Tested ✓
- Revisions ✓

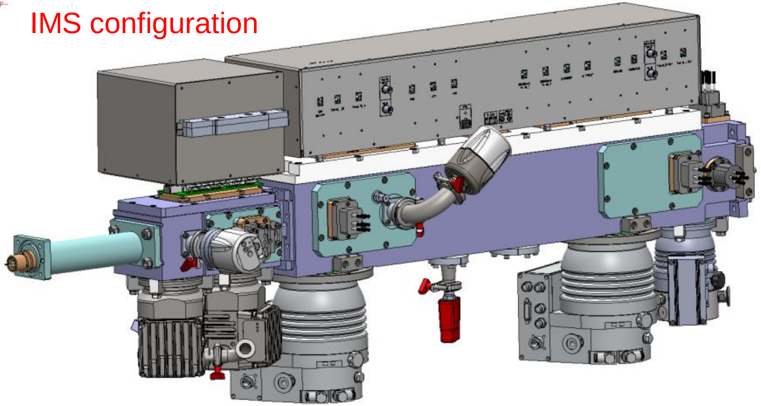
Omnitrap QExactive^{TM} HF



Omnitrap Exploris^{TM} 480

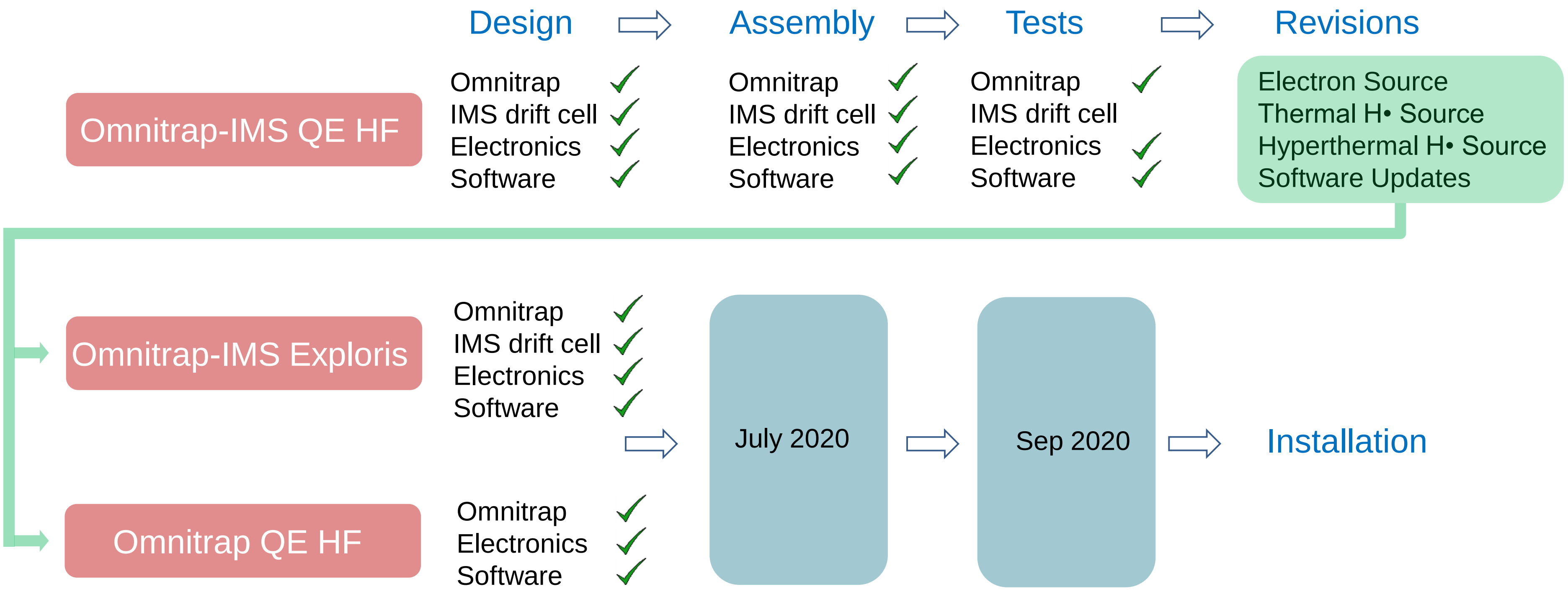


IMS configuration



Milestone: T1.2 – T1.5. Omnitraps & IMS Electronics design (FT, Dec 31, 2019)

Omnitrap Instrumentation Development Overview



Omnitrap-IMS QE HF

Design ⇨	Assembly ⇨	Tests ⇨
Omnitrap ✓	Omnitrap ✓	Omnitrap ✓
IMS drift cell ✓	IMS drift cell ✓	IMS drift cell
Electronics ✓	Electronics ✓	Electronics ✓
Software ✓	Software ✓	Software ✓

Revisions

- Electron Source
- Thermal H• Source
- Hyperthermal H• Source
- Software Updates

Omnitrap-IMS Exploris

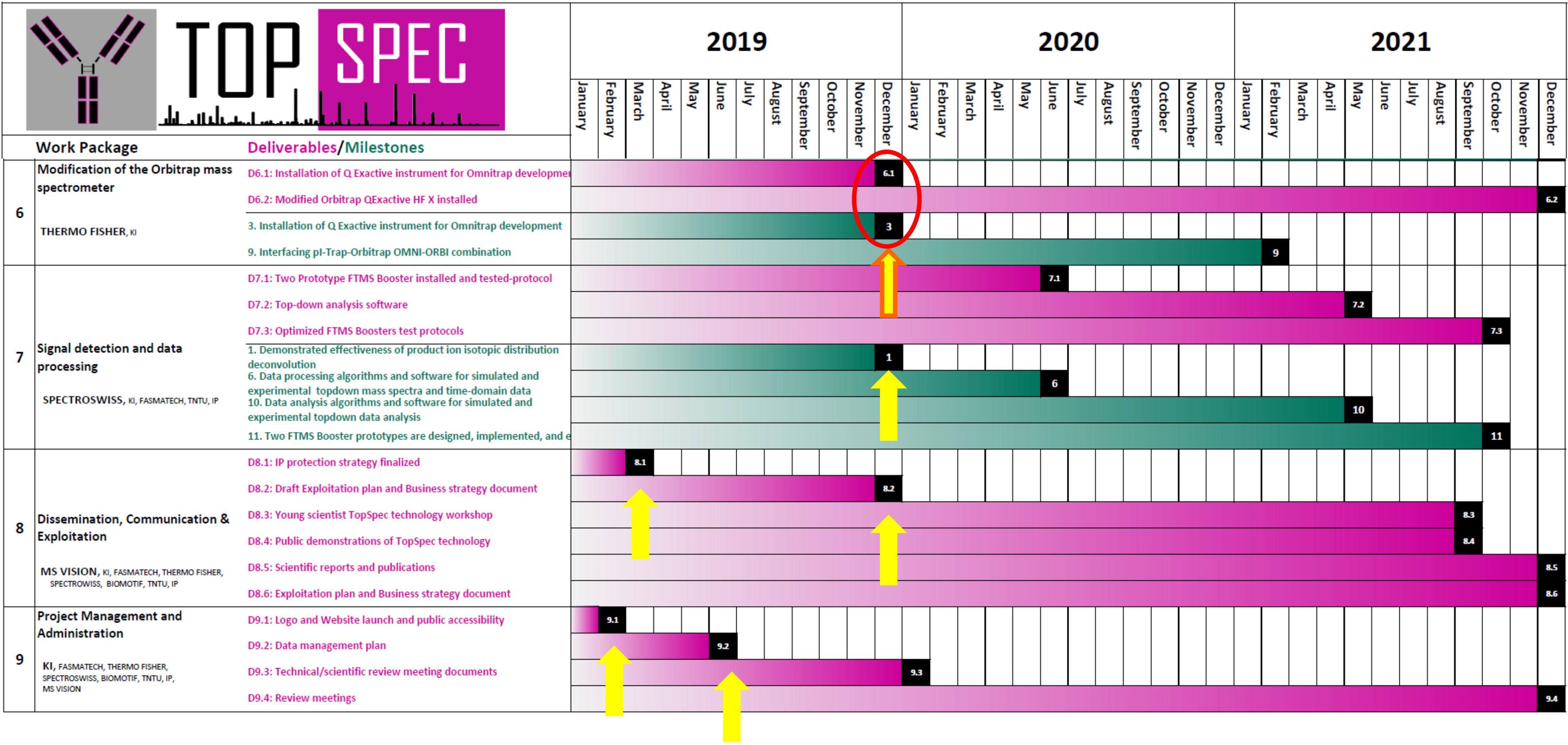
- Omnitrap ✓
- IMS drift cell ✓
- Electronics ✓
- Software ✓

Omnitrap QE HF

- Omnitrap ✓
- Electronics ✓
- Software ✓

⇨ July 2020 ⇨ Sep 2020 ⇨ Installation

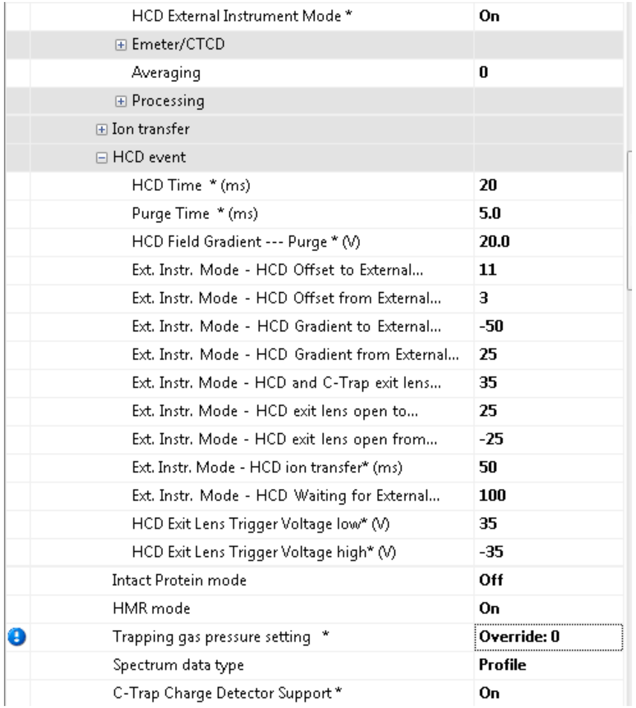
TopSpec Deliverables & Milestones



Work Package	Deliverables/Milestones	2019												2020												2021											
		January	February	March	April	May	June	July	August	September	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December
6 Modification of the Orbitrap mass spectrometer THERMO FISHER, KI	D6.1: Installation of Q Exactive instrument for Omnitrap developme												6.1																								
	D6.2: Modified Orbitrap QExactive HF X installed																																				6.2
	3. Installation of Q Exactive instrument for Omnitrap development												3																								
	9. Interfacing pI-Trap-Orbitrap OMNI-ORBI combination																										9										
7 Signal detection and data processing SPECTROSWISS, KI, FASMATECH, TNTU, IP	D7.1: Two Prototype FTMS Booster installed and tested-protocol																		7.1																		
	D7.2: Top-down analysis software																													7.2							
	D7.3: Optimized FTMS Boosters test protocols																																		7.3		
	1. Demonstrated effectiveness of product ion isotopic distribution deconvolution												1																								
	6. Data processing algorithms and software for simulated and experimental topdown mass spectra and time-domain data																		6																		
	10. Data analysis algorithms and software for simulated and experimental topdown data analysis																													10							
	11. Two FTMS Booster prototypes are designed, implemented, and e																																		11		
8 Dissemination, Communication & Exploitation MS VISION, KI, FASMATECH, THERMO FISHER, SPECTROWISS, BIOMOTIF, TNTU, IP	D8.1: IP protection strategy finalized			8.1																																	
	D8.2: Draft Exploitation plan and Business strategy document												8.2																								
	D8.3: Young scientist TopSpec technology workshop																																	8.3			
	D8.4: Public demonstrations of TopSpec technology																																	8.4			
	D8.5: Scientific reports and publications																																				8.5
	D8.6: Exploitation plan and Business strategy document																																				8.6
9 Project Management and Administration KI, FASMATECH, THERMO FISHER, SPECTROSWISS, BIOMOTIF, TNTU, IP, MS VISION	D9.1: Logo and Website launch and public accessibility		9.1																																		
	D9.2: Data management plan						9.2																														
	D9.3: Technical/scientific review meeting documents													9.3																							
	D9.4: Review meetings																																				9.4

Deliverable: D6.1. Installation of QE for Omnitrap development (FT, Dec 31, 2019)

QExactive$^{\text{TM}}$ HF Mass Spec installed in Athens (Sept 2019)

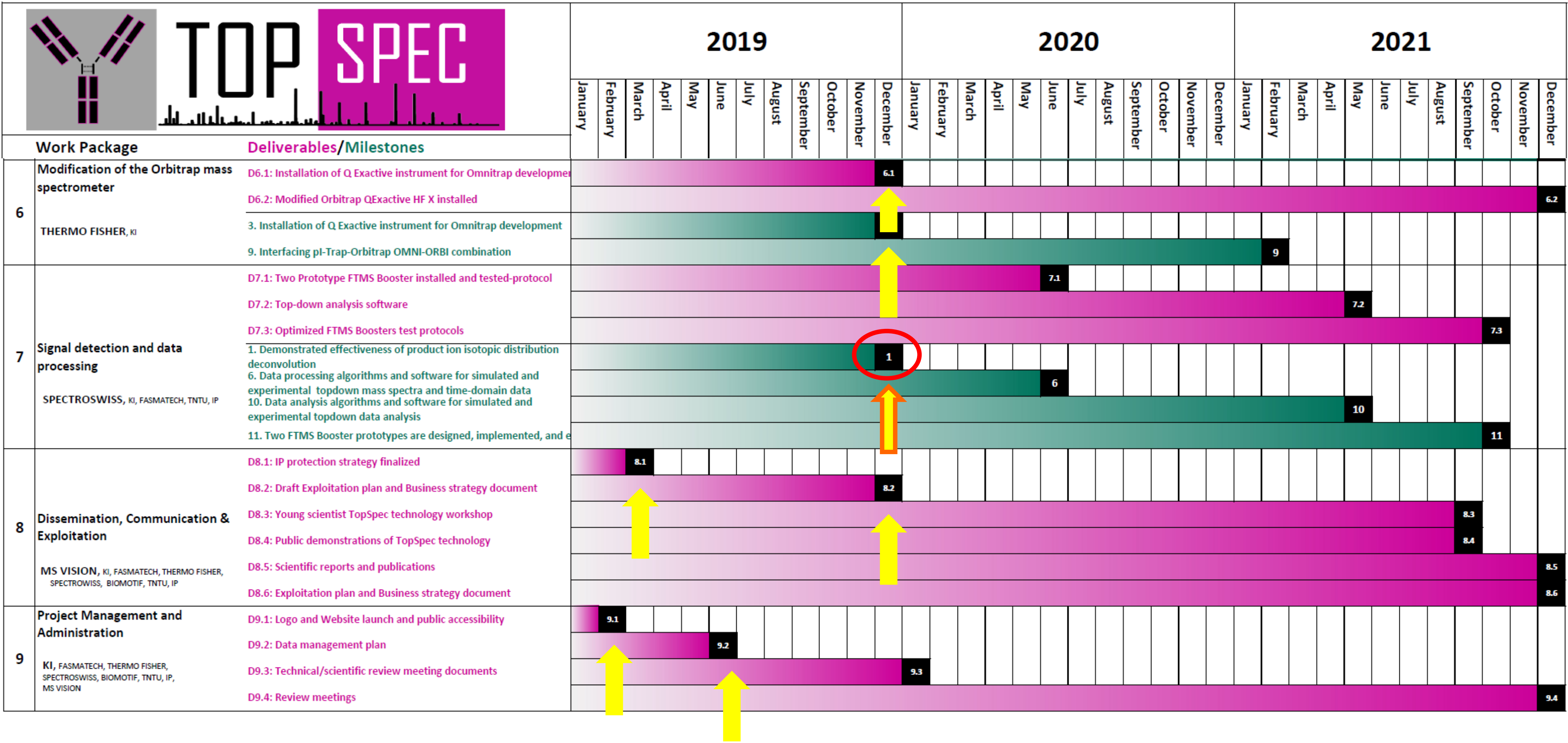


HCD External Instrument Mode *	On
⊞ Emeter/CTCD	
Averaging	0
⊞ Processing	
⊞ Ion transfer	
⊟ HCD event	
HCD Time * (ms)	20
Purge Time * (ms)	5.0
HCD Field Gradient --- Purge * (V)	20.0
Ext. Instr. Mode - HCD Offset to External...	11
Ext. Instr. Mode - HCD Offset from External...	3
Ext. Instr. Mode - HCD Gradient to External...	-50
Ext. Instr. Mode - HCD Gradient from External...	25
Ext. Instr. Mode - HCD and C-Trap exit lens...	35
Ext. Instr. Mode - HCD exit lens open to...	25
Ext. Instr. Mode - HCD exit lens open from...	-25
Ext. Instr. Mode - HCD ion transfer* (ms)	50
Ext. Instr. Mode - HCD Waiting for External...	100
HCD Exit Lens Trigger Voltage low* (V)	35
HCD Exit Lens Trigger Voltage high* (V)	-35
Intact Protein mode	Off
HMR mode	On
Trapping gas pressure setting *	Override: 0
Spectrum data type	Profile
C-Trap Charge Detector Support *	On

Tune s/w modifications ✓

Debugging ✓

TopSpec Deliverables & Milestones



			2019												2020												2021											
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		D6.2: Modified Orbitrap QExactive HF X installed																																				6.2
		3. Installation of Q Exactive instrument for Omnitrap development																																				
		9. Interfacing pI-Trap-Orbitrap OMNI-ORBI combination																										9										
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		D7.2: Top-down analysis software																													7.2							
		D7.3: Optimized FTMS Boosters test protocols																																		7.3		
		1. Demonstrated effectiveness of product ion isotopic distribution deconvolution												1																								
		6. Data processing algorithms and software for simulated and experimental topdown mass spectra and time-domain data																		6																		
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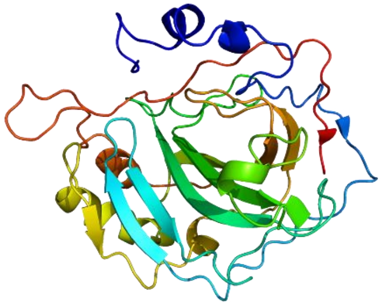
9	Project Management and Administration KI, FASMATECH, THERMO FISHER, SPECTROSWISS, BIOMOTIF, TNTU, IP, MS VISION	D9.1: Logo and Website launch and public accessibility		9.1																																		
		D9.2: Data management plan						9.2																														
		D9.3: Technical/scientific review meeting documents													9.3																							
		D9.4: Review meetings																																				9.4

Milestone: T7.1. Demonstrated ion isotopic distribution deconvolution (SPS, Dec 31, 2019)

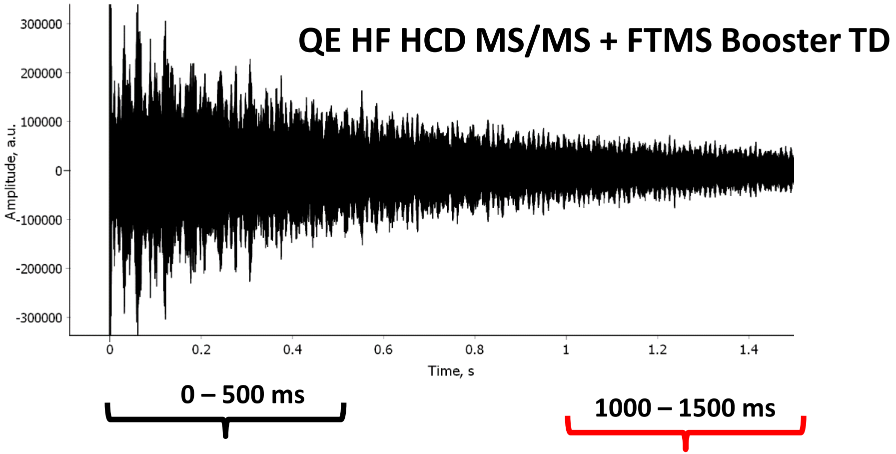
Hypothesis: Information on product ion signal decay rate could help deconvolve complex top-down spectra

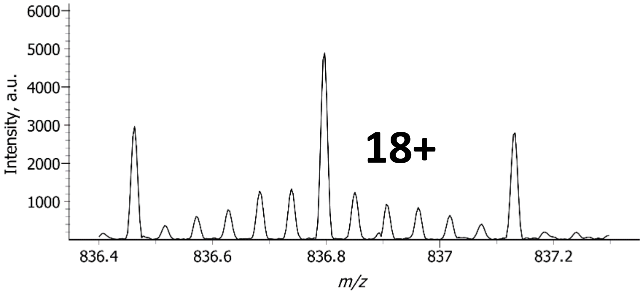
Milestone M1: mobility-like resolution of ~**7.1** is achieved, further improvements are being researched

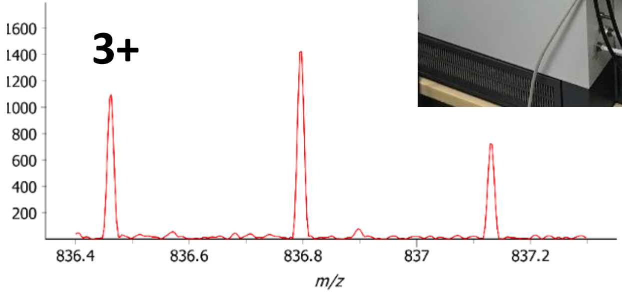


Carbonic anhydrase

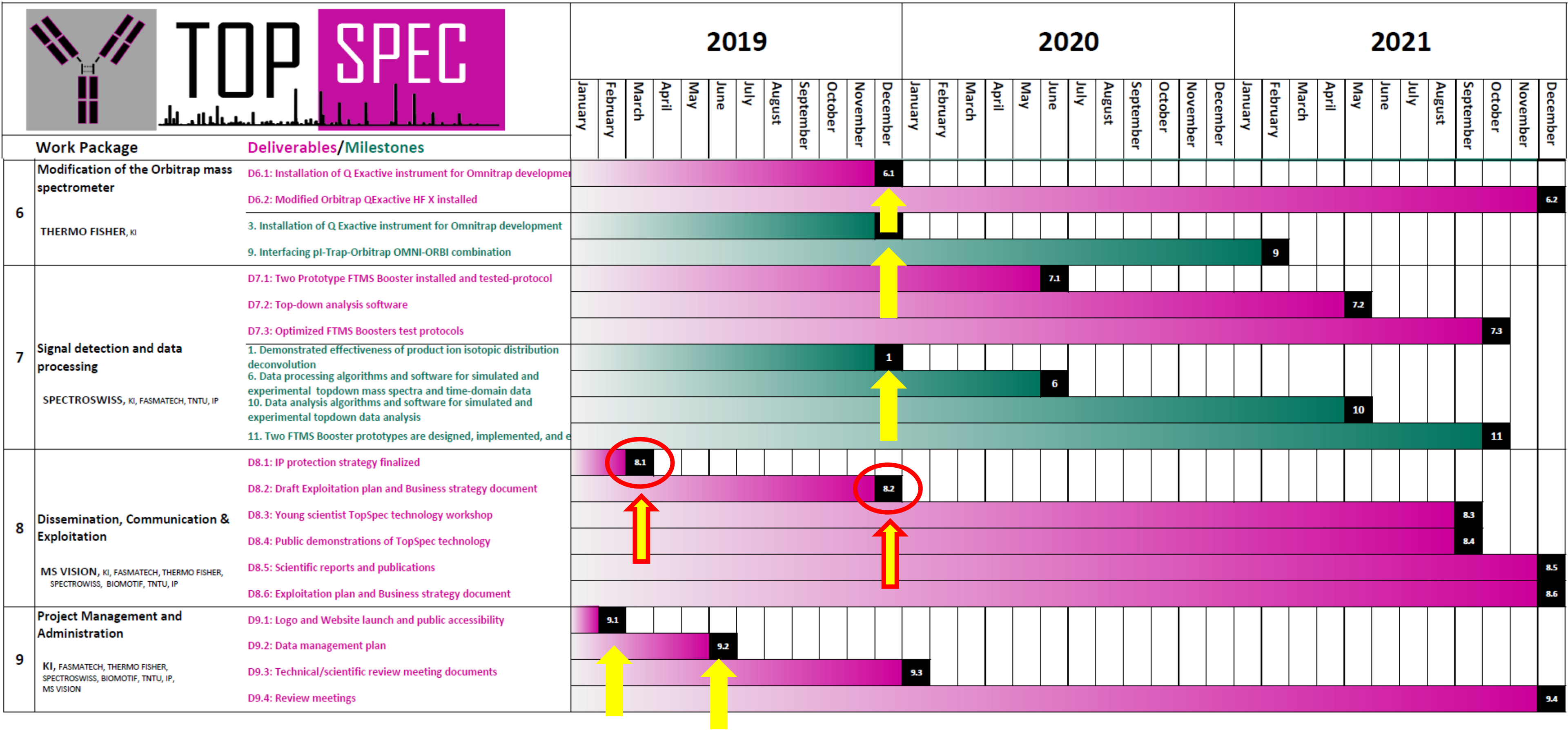
29 kDa







TopSpec Deliverables & Milestones



Work Package	Deliverables/Milestones	2019	2020	2021
6 **Modification of the Orbitrap mass spectrometer** THERMO FISHER, KI	D6.1: Installation of Q Exactive instrument for Omnitrap developme	December 6.1		
	D6.2: Modified Orbitrap QExactive HF X installed			December 6.2
	3. Installation of Q Exactive instrument for Omnitrap development	December		
	9. Interfacing pI-Trap-Orbitrap OMNI-ORBI combination			February 9
7 **Signal detection and data processing** SPECTROSWISS, KI, FASMATECH, TNTU, IP	D7.1: Two Prototype FTMS Booster installed and tested-protocol		June 7.1	
	D7.2: Top-down analysis software			May 7.2
	D7.3: Optimized FTMS Boosters test protocols			October 7.3
	1. Demonstrated effectiveness of product ion isotopic distribution deconvolution	December 1		
	6. Data processing algorithms and software for simulated and experimental topdown mass spectra and time-domain data		June 6	
	10. Data analysis algorithms and software for simulated and experimental topdown data analysis			May 10
	11. Two FTMS Booster prototypes are designed, implemented, and e			October 11
8 **Dissemination, Communication & Exploitation** MS VISION, KI, FASMATECH, THERMO FISHER, SPECTROWISS, BIOMOTIF, TNTU, IP	D8.1: IP protection strategy finalized	March 8.1		
	D8.2: Draft Exploitation plan and Business strategy document	December 8.2		
	D8.3: Young scientist TopSpec technology workshop			September 8.3
	D8.4: Public demonstrations of TopSpec technology			September 8.4
	D8.5: Scientific reports and publications			December 8.5
	D8.6: Exploitation plan and Business strategy document			December 8.6
9 **Project Management and Administration** KI, FASMATECH, THERMO FISHER, SPECTROSWISS, BIOMOTIF, TNTU, IP, MS VISION	D9.1: Logo and Website launch and public accessibility	February 9.1		
	D9.2: Data management plan	June 9.2		
	D9.3: Technical/scientific review meeting documents		January 9.3	
	D9.4: Review meetings			December 9.4

Deliverable: D8.1 IP protection strategy (MSV, Mar 31, 2019)

Horizon 2020

Grant Agreement no: 829157

TopSpec

Project Deliverable Report

D8.1 TopSpec Intellectual Property Protection Strategy

Version: 1.2
Author: Jan Commandeur (MS)
Contributors: -
Internal reviewers: -
Deliverable due date: 2019-04-01
Actual submission date:

Work package: WP8
Task: T8.5
Dissemination level: Confidential (CO)*
Lead beneficiary: MS
Status: Progress

D8.1 IP protection strategy

Created ✓
Reviewed ✓
Submitted ✓

Covers:

- IP Strategy and Policy
- Use and ownership IP
- Background / foreground IP
- IP Asset Register

Deliverable: D8.2: Draft Exploitation plan & Business strategy (MSV, Dec 31, 2019)

TOP SPEC Horizon 2020

Grant Agreement no: 829157

TopSpec

Project Deliverable Report

D8.2 Draft Exploitation and Dissemination Plan

Version:	1.1
Author:	Remco Swart (MS)
Contributors:	Susanna Lundström (KI), Jan Commandeur (MS)
Internal reviewers:	-
Deliverable due date:	2019-12-31
Actual submission date:	2019-12-19

Work package:	WP8
Task:	T8.2
Dissemination level:	Public
Lead beneficiary:	MS
Status:	Progress

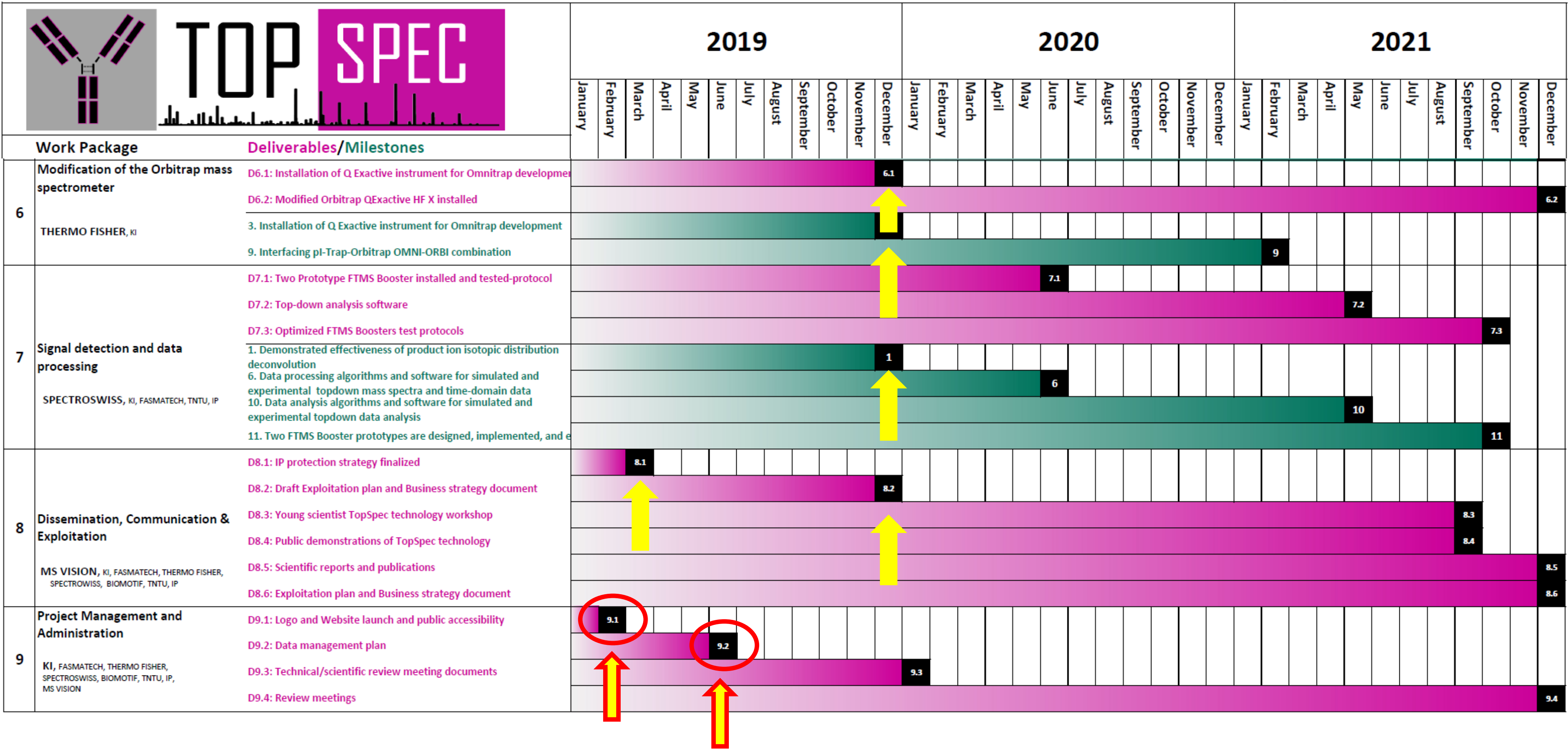
<ins>D8.2 Draft Exploitation plan & business strategy</ins>

Created	✓
Reviewed	✓
Submitted	✓

Covers:

- Tools for tracking activities
- Marketing collaterals
- Social media communication
- Meetings and conferences
- Interaction with partners
- Connect with potential users
- Exploitation of results

TopSpec Deliverables & Milestones



		2019												2020												2021											
Work Package	Deliverables/Milestones	January	February	March	April	May	June	July	August	September	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December
6 **Modification of the Orbitrap mass spectrometer** **THERMO FISHER**, KI	D6.1: Installation of Q Exactive instrument for Omnitrap developme												6.1																								
	D6.2: Modified Orbitrap QExactive HF X installed																																				6.2
	3. Installation of Q Exactive instrument for Omnitrap development																																				
	9. Interfacing pI-Trap-Orbitrap OMNI-ORBI combination																										9										
7 **Signal detection and data processing** **SPECTROSWISS**, KI, FASMATECH, TNTU, IP	D7.1: Two Prototype FTMS Booster installed and tested-protocol																		7.1																		
	D7.2: Top-down analysis software																													7.2							
	D7.3: Optimized FTMS Boosters test protocols																																		7.3		
	1. Demonstrated effectiveness of product ion isotopic distribution deconvolution												1																								
	6. Data processing algorithms and software for simulated and experimental topdown mass spectra and time-domain data																		6																		
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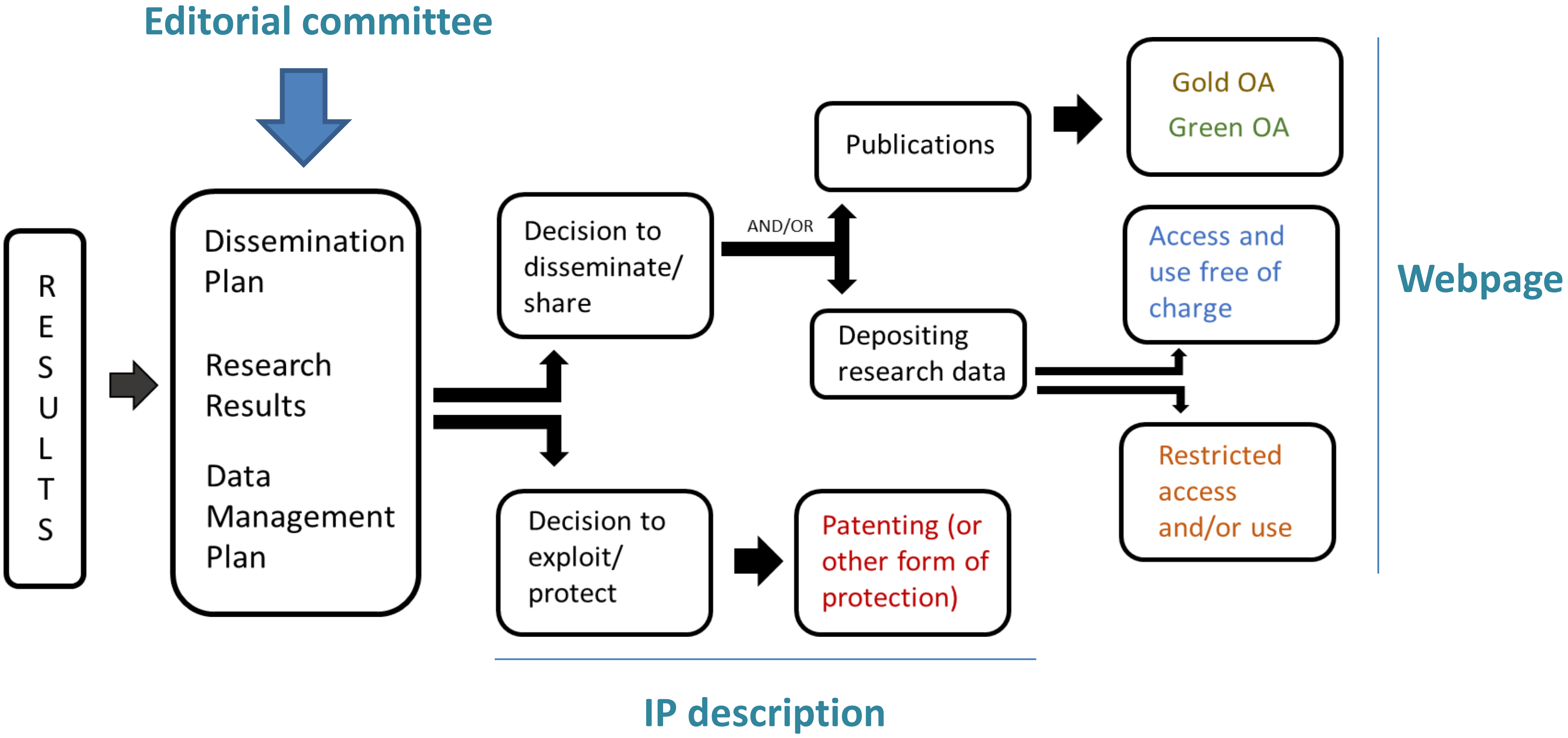
	11. Two FTMS Booster prototypes are designed, implemented, and e																																		11		
8 **Dissemination, Communication & Exploitation** **MS VISION**, KI, FASMATECH, THERMO FISHER, SPECTROWISS, BIOMOTIF, TNTU, IP	D8.1: IP protection strategy finalized			8.1																																	
	D8.2: Draft Exploitation plan and Business strategy document												8.2																								
	D8.3: Young scientist TopSpec technology workshop																																	8.3			
	D8.4: Public demonstrations of TopSpec technology																																	8.4			
	D8.5: Scientific reports and publications																																				8.5
	D8.6: Exploitation plan and Business strategy document																																				8.6
9 **Project Management and Administration** **KI**, FASMATECH, THERMO FISHER, SPECTROSWISS, BIOMOTIF, TNTU, IP, MS VISION	D9.1: Logo and Website launch and public accessibility		9.1																																		
	D9.2: Data management plan						9.2																														
	D9.3: Technical/scientific review meeting documents													9.3																							
	D9.4: Review meetings																																				9.4

Deliverable D9.1 Logo and Website launch and public accessibility (KI, Feb 28, 2019)

Logo:

Website: https://topspec.ki.se



Editorial committee

RESULTS

Dissemination Plan

Research Results

Data Management Plan

Decision to disseminate/ share

AND/OR

Publications

Gold OA

Green OA

Depositing research data

Access and use free of charge

Restricted access and/or use

Decision to exploit/ protect

Patenting (or other form of protection)

Webpage

IP description

Summary:

All deliverables and milestones have been reached by the TopSpec Consortium

in full and in time

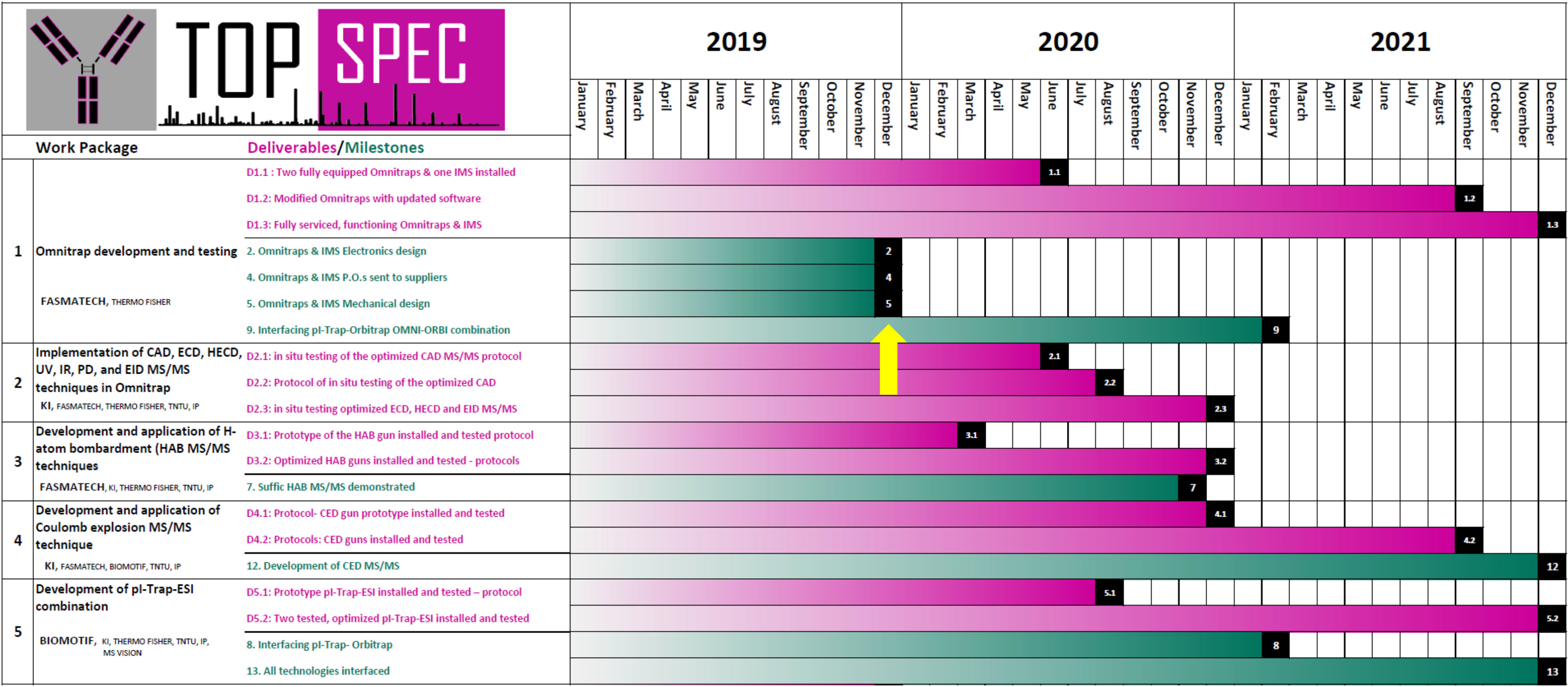
Prospects for 2020-2021

- No major deviation from the working plan content is foreseen
- No major deviation from the time schedule is foreseen

Unplanned findings useful for TopSpec

- Tandem pI-fractionation promises higher resolution in shorter time
- Deconvolution based on transient decay seems to exceed expectations, reaching the resolution 6-7 instead of 2-3.

TopSpec Deliverables & Milestones



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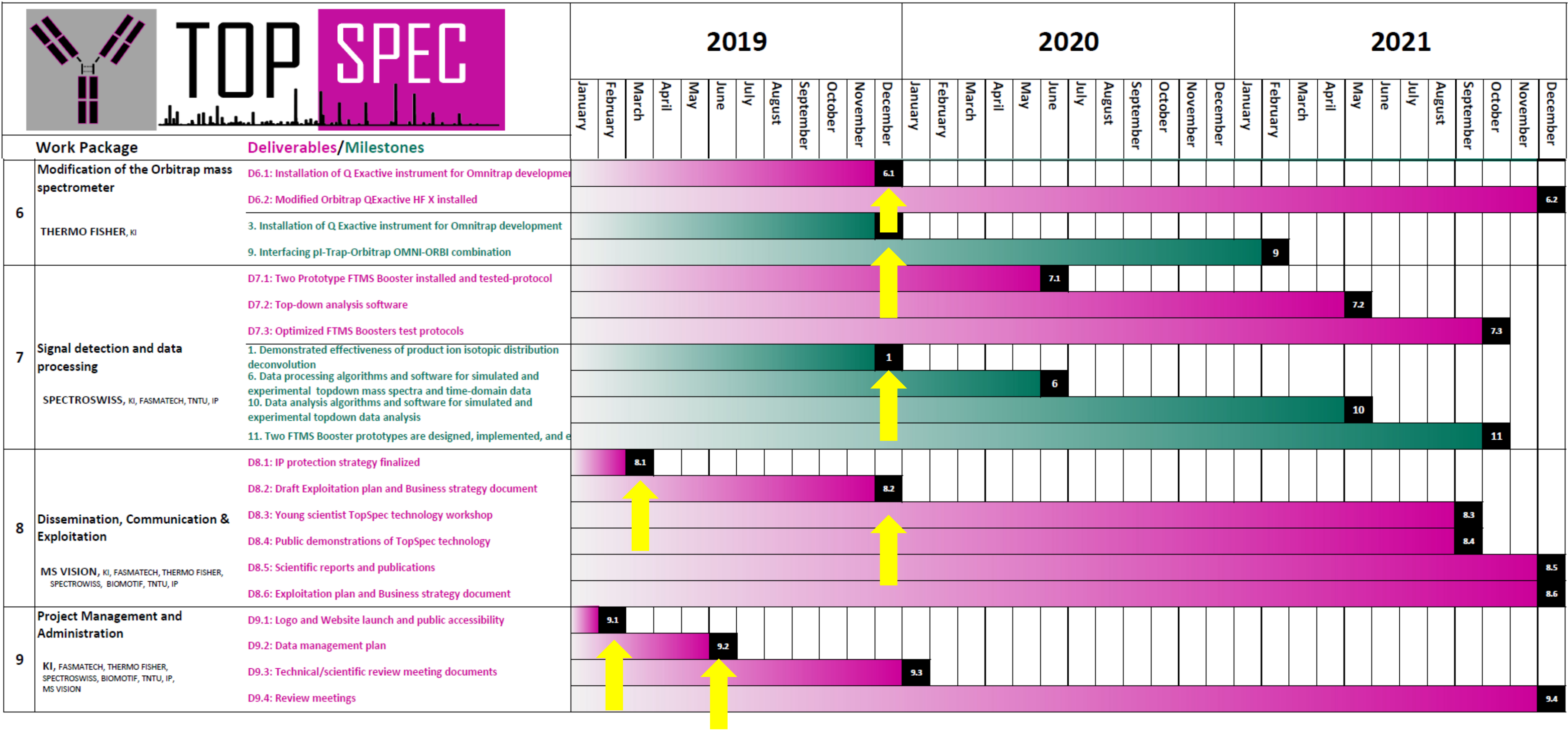
1	Omnitrap development and testing FASMATECH, THERMO FISHER	D1.1 : Two fully equipped Omnitraps & one IMS installed																		1.1																		
		D1.2: Modified Omnitraps with updated software																																	1.2			
		D1.3: Fully serviced, functioning Omnitraps & IMS																																				1.3
		2. Omnitraps & IMS Electronics design												2																								
		4. Omnitraps & IMS P.O.s sent to suppliers												4																								
		5. Omnitraps & IMS Mechanical design												5																								
		9. Interfacing pI-Trap-Orbitrap OMNI-ORBI combination																										9										
2	Implementation of CAD, ECD, HECD, UV, IR, PD, and EID MS/MS techniques in Omnitrap KI, FASMATECH, THERMO FISHER, TNTU, IP	D2.1: in situ testing of the optimized CAD MS/MS protocol																		2.1																		
		D2.2: Protocol of in situ testing of the optimized CAD																				2.2																
		D2.3: in situ testing optimized ECD, HECD and EID MS/MS																								2.3												
3	Development and application of H-atom bombardment (HAB MS/MS techniques FASMATECH, KI, THERMO FISHER, TNTU, IP	D3.1: Prototype of the HAB gun installed and tested protocol															3.1																					
		D3.2: Optimized HAB guns installed and tested - protocols																								3.2												
		7. Suffic HAB MS/MS demonstrated																							7													
4	Development and application of Coulomb explosion MS/MS technique KI, FASMATECH, BIOMOTIF, TNTU, IP	D4.1: Protocol- CED gun prototype installed and tested																								4.1												
		D4.2: Protocols: CED guns installed and tested																																	4.2			
		12. Development of CED MS/MS																																				12
5	Development of pI-Trap-ESI combination BIOMOTIF, KI, THERMO FISHER, TNTU, IP, MS VISION	D5.1: Prototype pI-Trap-ESI installed and tested – protocol																				5.1																
		D5.2: Two tested, optimized pI-Trap-ESI installed and tested																																				5.2
		8. Interfacing pI-Trap- Orbitrap																										8										
		13. All technologies interfaced																																				13

TopSpec Deliverables & Milestones



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