Cutting-Edge Multi-Level Analytical and Structural Characterization of Antibody-Based Therapeutics

Alain BECK - AT Europe Lisbon - May 23, 2022 (90)

Cutting-Edge Multi-Level Analytical & Structural Characterization of Antibody-Based Therapeutics

(1) Introduction:

- Therapeutic mAbs & related products (BsAbs, Fc-fusion prot., ADCs)
- Analytical & structural toolbox and newtwork (2012-2022)
- Analytical workflow (Top down, Middle up/down, Bottom up)

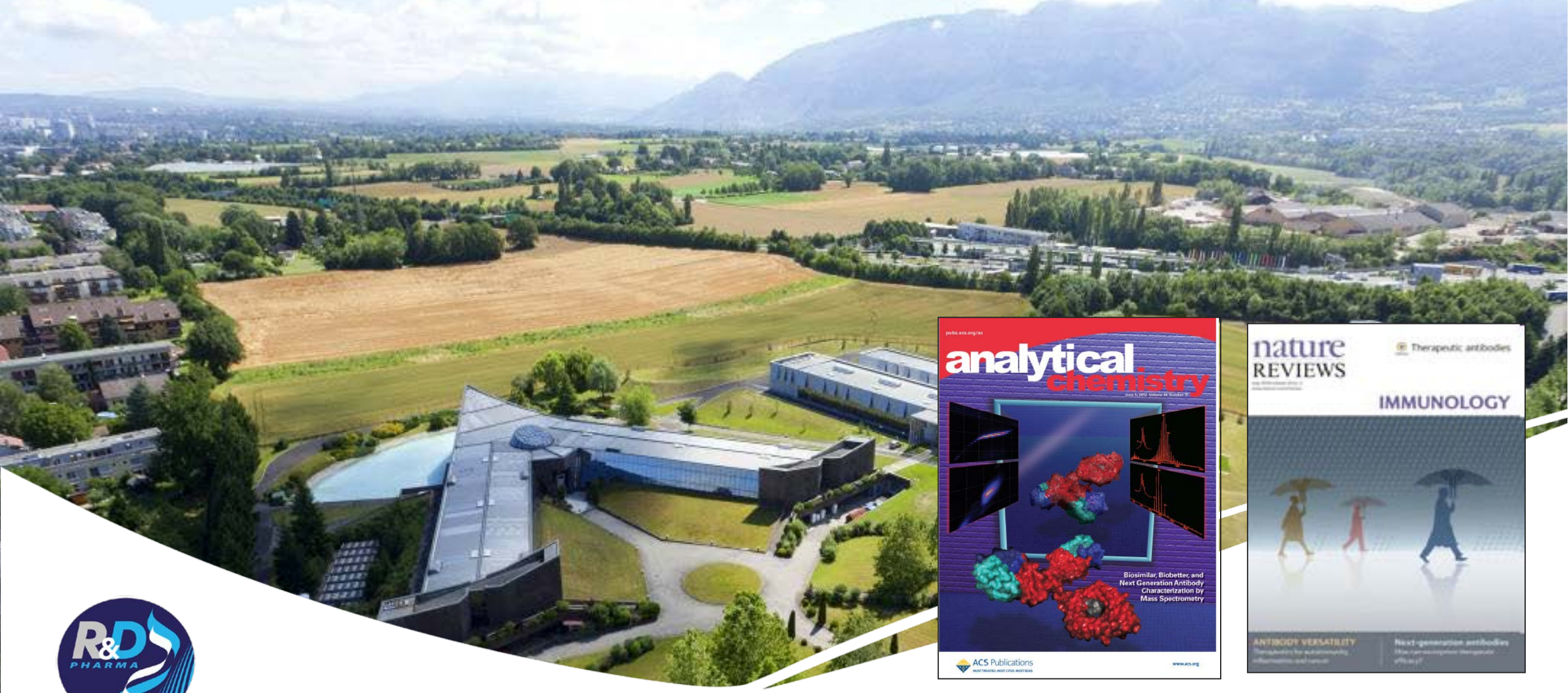
(2) Monoclonal antibody structure & Critical Quality Attributes (Biosimilars)

- Cys-related, Size, Oxidized, Glyco & Charged variants assessment

(3) State of the art analytical methods for

- BsAbs,
- Fc-fusion proteins & peptides,
- ADCs

(4) Summary & Take-home messages (Covid-19 pandemic and beyond)



(1) Introduction: Therapeutic mAbs & related products

- Analytical & Structural Toolbox (2012-2022)
- Analytical & Structural Workflow (Top, Middle, Bottom)

Pierre Fabre

+119 antibody-based therapeutics FDA/EMA appr. (2021)

5/5/2021 FDA approves 100th monoclonal antibody product

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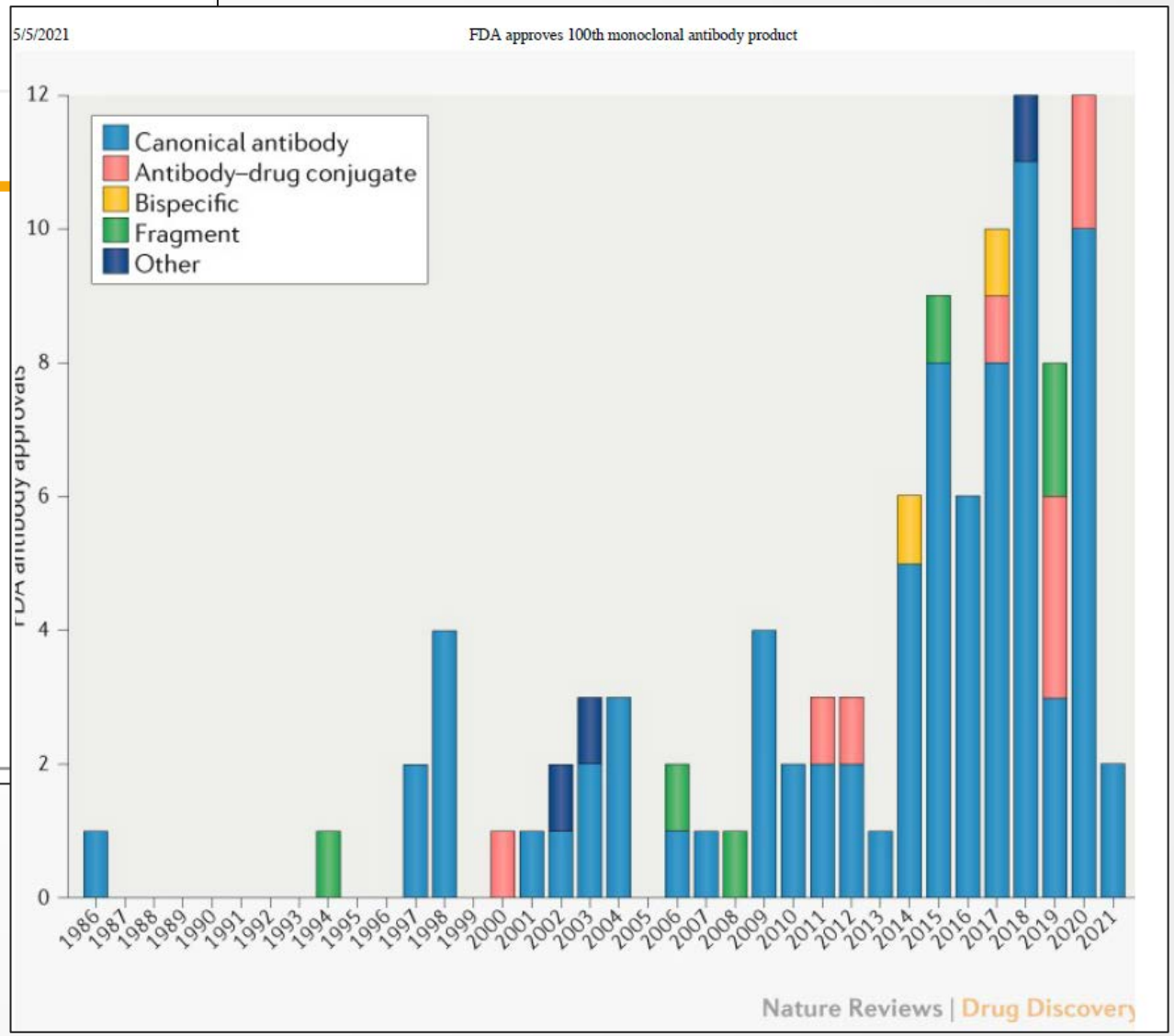
NEWS · 05 MAY 2021

FDA approves 100th monoclonal antibody product

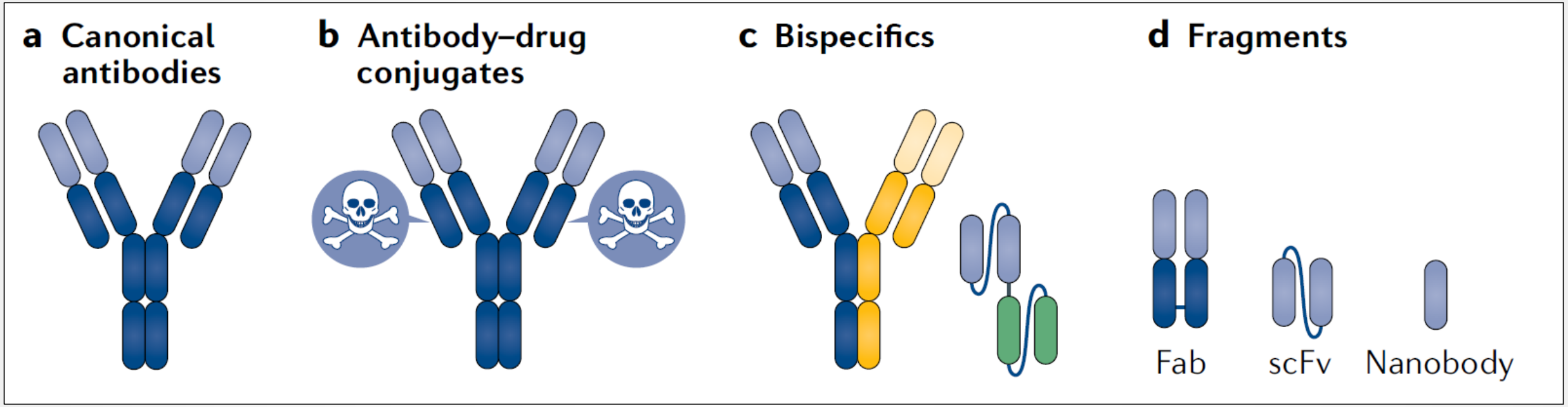
Thirty-five years on from the FDA's approval of a first monoclonal antibody, these biologics account for nearly a fifth of the agency's new drug approvals each year.

Asher Mullard



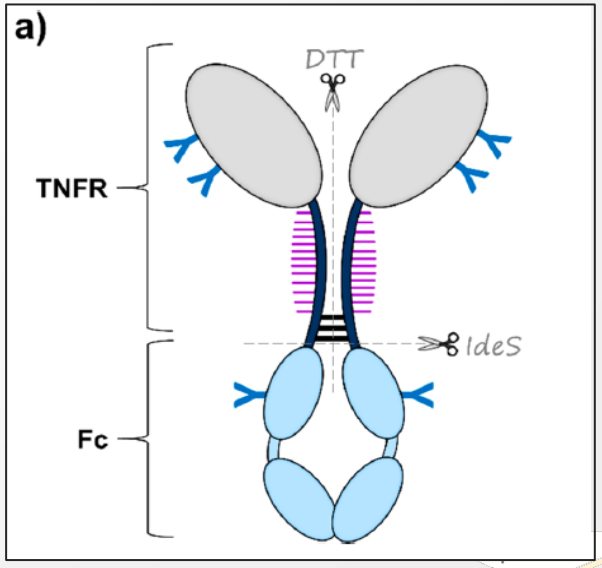
- Antibodies/ Fc-fusion proteins (2020) :
  - 11/20 top therapeutics by sales
  - 184 billions USD
- +14 FDA approved in 2021
- 870 antibodies in clinical development

+119 antibody-based therapeutics FDA/EMA appr.



+14 Fc-fusion proteins FDA/EMA appr.

(3/20 top therapeutics by sales (2020))



mAbs: analytical & structural toolbox (2012/2013)

Analytical Chemistry — Review

MAb primary structure assessment

Charge variants
- Separation techniques
  - IEF, cIEF, icIEF
  - HPLC (IEX, RP, HIC)
  - Boronate affinity chrom.
- Mass spectrometry
  - Middle-up LC-MS
  - Peptide mapping (LC-MS/MS)
  - Top-down MS/MS

AA sequence and variants
- Intact mass (ESI-MS)
  - Glycan removal (PNGase F)
- Bottom-up peptide mapping
  - Enzyme digestion and LC-MS/MS
- Middle-up (LC-MS)
  - Red. mAb (light and heavy chains)
  - Limited proteolysis (IdeS, papain) + reduction (25 kDa fragments)
- Top/middle-down (HR-MS/MS)
  - ETD/ECD and CID
- SEC, CE-SDS

Glycovariants
- Glycan (released)
  - CE-LIF
  - HPLC (NP, HILIC, ZIC-HILIC, IEX, PGC)
  - MALDI-TOF, ESI-MS and MS/MS
  - Electronic impact-MS (with GC)
- Glyco-protein/ peptide
  - Intact/ middle-up LC-MS
  - Peptide mapping (LC-MS and MS/MS)

Cysteine-linked variants
- Ellman assay (free Cys)
- Differential peptide mapping
  - > red/ and non-red cond.
  - > CID and ETD (Cys linkage)
- IM-MS (Cys linkage)

Higher order structures, aggregates and mAb/Ag

Higher order
- XRD
- Native-MS
- IM-MS
- HDX-MS

Aggregates
- SEC (UV-MALS)
- A4F (UV-MALS)
- AUC
- Native MS
- IM-MS
- HDX-MS
- Crosslinking MS

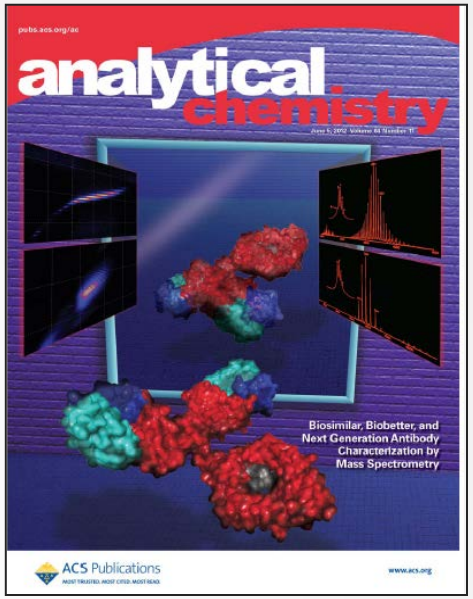
mAb/Ag complexes
- SPR
- ELISA
- FACS
- Native MS
- IM-MS
- HDX-MS
- Crosslinking MS

PK/ Quantification

- ELISA
- Radioimmuno-assay
- Immunofluorescence
- Isotope dilution – SRM
- Isotope dilution LC-MS

- **Main proteoforms**
- **Higher Order Structures**

CQAs:
- **Cys-relat. variants**
- **Size variants**
- **Oxidized variants**
- **Glyco-variants**
- **Charge variants**



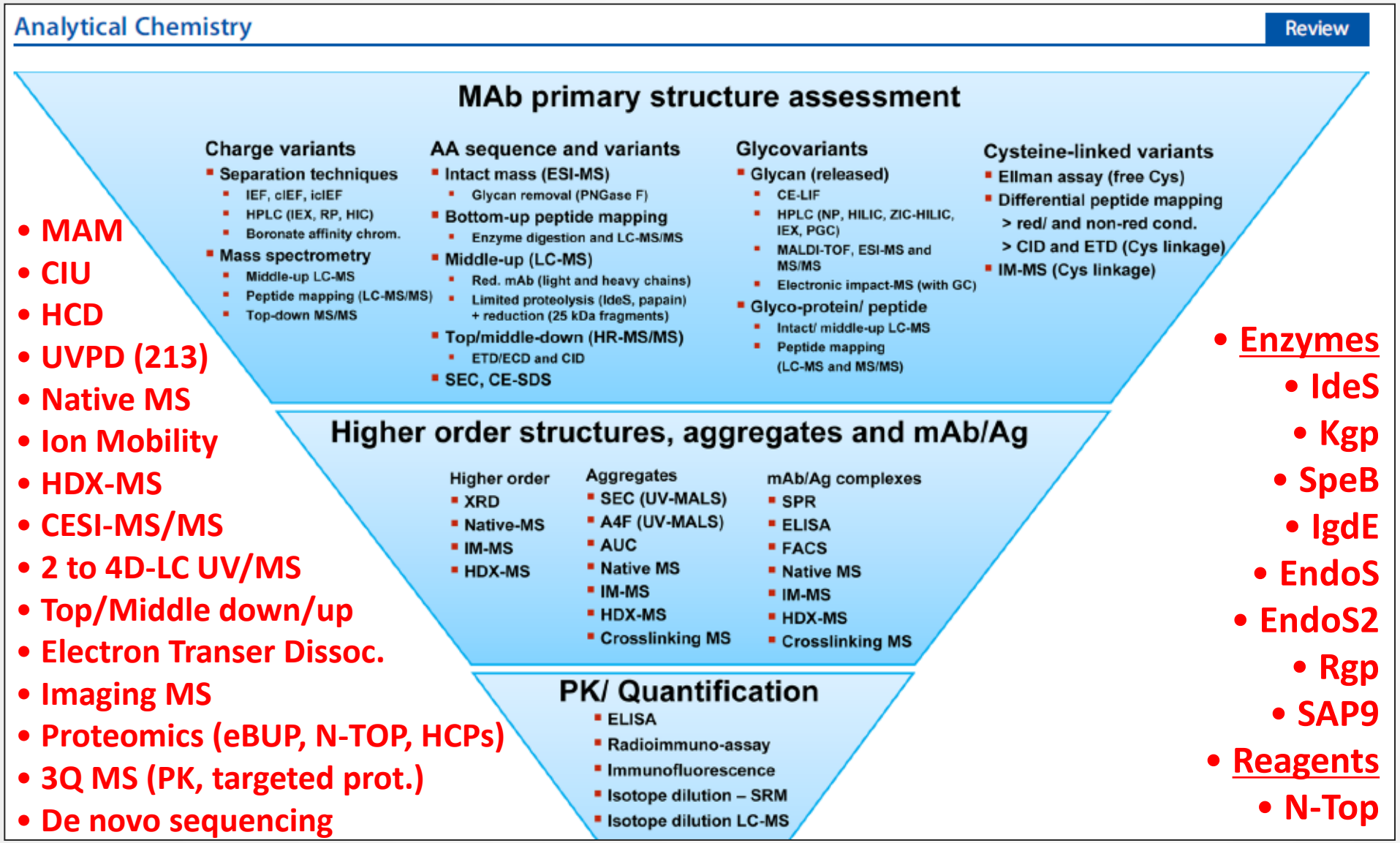
- S. Cianferani
- A. Van Dorsselaer and coll.

(LSMBO, University of Strasbourg)

IF: 6.350
545 citations

➢ **Beck A, Wagner E, Ayoub D, Van Dorsselaer A, Cianferani S. Anal Chem 2013**

mAbs: analytical & structural methods (2022)



- MAM
- CIU
- HCD
- UVPD (213)
- Native MS
- Ion Mobility
- HDX-MS
- CESI-MS/MS
- 2 to 4D-LC UV/MS
- Top/Middle down/up
- Electron Transer Dissoc.
- Imaging MS
- Proteomics (eBUP, N-TOP, HCPs)
- 3Q MS (PK, targeted prot.)
- De novo sequencing

- Enzymes
  - IdeS
  - Kgp
  - SpeB
  - IgdE
  - EndoS
  - EndoS2
  - Rgp
  - SAP9
- Reagents
  - N-Top

- S. Cianferani
- D. Guillarme
- JL. Veuthey
- Y. François
- S. Heinisch
- Y. Tsybin
- D. Stoll
- A. Delobel
- J. Sjögren
- W. Chen
- D. Suckau
- H. Liu & coll.

GENOVIS

➢ Beck A, Wagner E, Ayoub D, Van Dorsselaer A, Cianferani S. Anal Chem 2013

Analytical and structural workflow (2012-2022)

MASS SPECTROMETRY

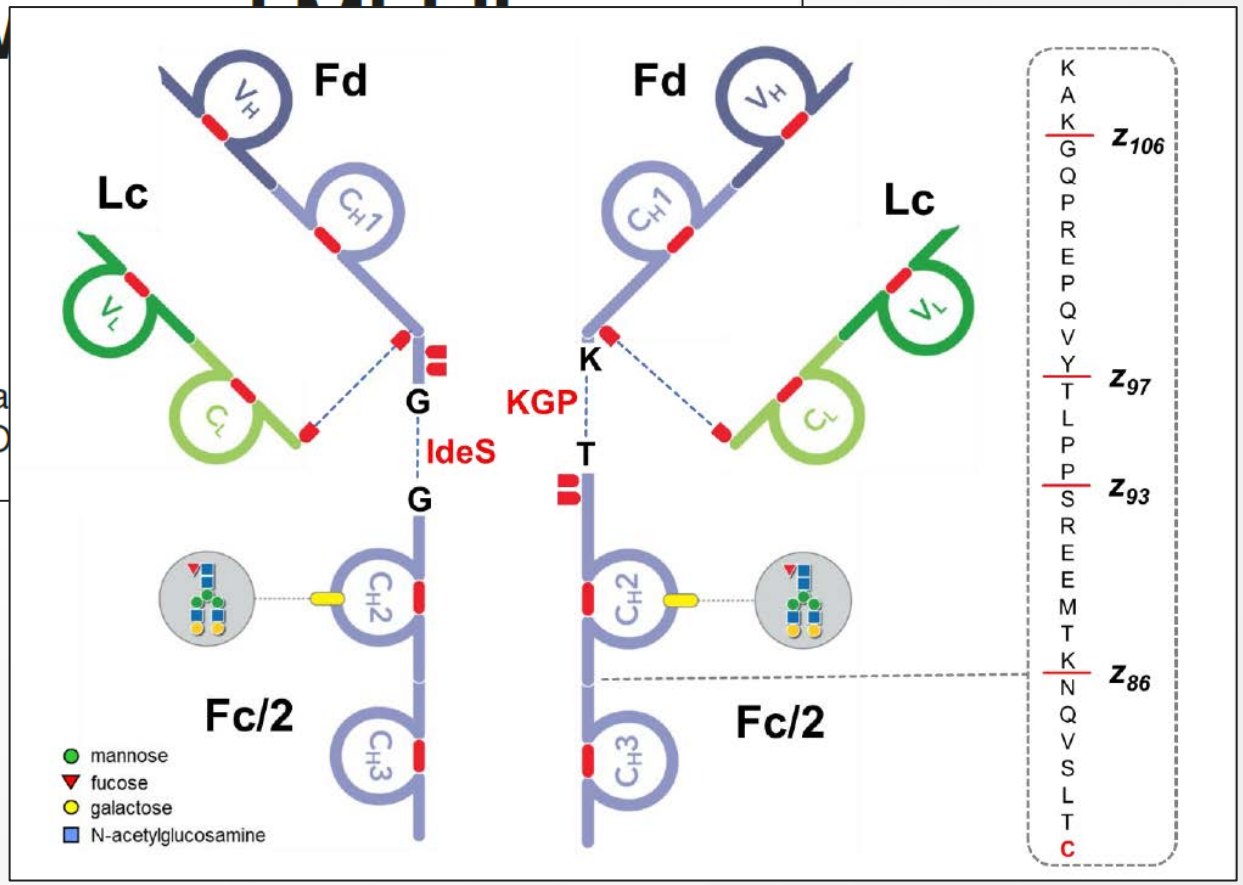
CHIMIA 2022, 76, No. 1/2 1

doi:10.2533/chimia.2022.1

Chimia 76 (2022) 1–13 © Y.O. Tsybin* et al.

Structural Analysis of Monoclonal Antibodies with Top-dow down Electron Transfer Spectrometry: The First

Luca Fornelli[a], Daniel Ayoub[b], Kristina Srzentić[cf], Konsta
Natalia Gasilova[c], Laure Menin[c], Alain Beck[e], and Yury O



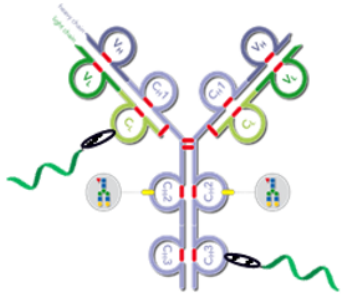
- Y. Tsybin et al

- Fornelli, L, Ayoub D, Beck A, Tsybin Y et al, Chimia 2022

Analytical and structural workflow (2012-2022)

TOP-DOWN, INTACT

> 150 kDa



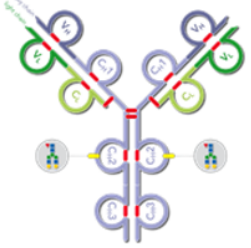
Species: intact ADCs and AOCs

Sample preparation: none, desalting, deglycosylation

CQAs: major glycosylations, DARs, PTMs, integrity

Methods: **intact mass** measurements by native and denaturing MS via c

~ 150 kDa



Species: intact antib

Sample preparation

CQAs: major glycos

Methods: **intact ma** denaturing MS via c **top-down** analysis

MIDDLE-UP

~ 100 kDa

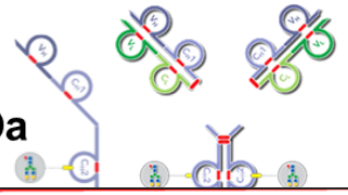
Species: mAb/IgG subunits ($F(ab)_2$)

Sample preparation: structure-specific digestion (IdeS)

CQAs: limited sequencing, major glycosylations, PTMs

Methods: **middle-up** mass measurements by direct infusion, RPLC-MS, SEC-MS, …
middle-down analysis with HCD/ETD/UVPD, …

~ 50 kDa



Species: mAb/IgG subunits (F(ab), Fc, and Hc)

Sample preparation: S-S bond reduction, structure-specific digestion (KGP, IdeS, papain, etc.)

CQAs: chain pairing, limited sequencing, large PTMs

Methods: **middle-up** mass measurements : direct …ion and RPLC-MS; **middle-down** analysis with …/ETD/UVPD, …

…ies: mAb/IgG subunits (Lc, Fd, and Fc/2)

…ple preparation: structure-specific digestion (IdeS, …, papain, etc.) followed by S-S bond reduction

…s: limited sequencing, PTMs (oxidation, glycation, etc.)

…ods: **middle-up** mass measurements (direct …ion and RPLC-MS); **middle-down** analysis with …/ETD/UVPD, …

BOTTOM-UP

< 10 kDa

Species: proteolityc mAb/IgG fragments (peptides)

Sample preparation: sequence specific and non-specific enzymes (trypsin, chymotrypsin, Lys-C, Sap9, etc.)

CQAs: complete sequencing (all CDRs), all PTMs (deamidation, oxidation, etc.)

Methods: RPLC-MS (extended) **bottom-up** with HCD/ETD/UVPD, …

Spectroswiss

- Fornelli L, Tsybin Y et al, Chimia 2022

Interlaboratory Study for Characterizing mAbs by Top-Down and Middle-Down Mass Spec (JASMS 2020)

Journal of the American Society for Mass Spectrometry

pubs.acs.org/jasms

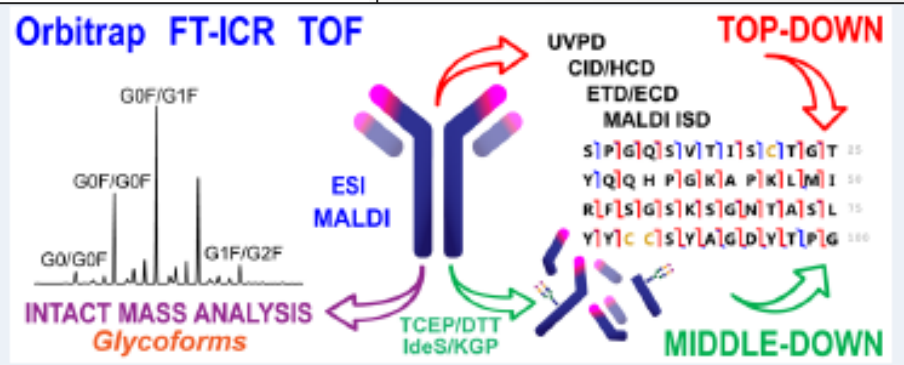
- Y. Tsybin et al

Interlaboratory Study Top-Down and Middle

Kristina Srzentić,† Luca Fornelli,†
Lissa C. Anderson, Dina L. Bai, A
Julia Chamot-Rooke, Sneha Chatt
Robert A. D'Ippolito, Mathieu Du
Sylvester Greer, Kim F. Haselman
Matthew V. Holt, Sam Hughes, D
Christian Malosse, Alan G. Marsh
Simone Nicolardi, Ljiljana Paša-T
Wendy Sandoval, Richa Sarin, Ni
Michael R. Shortreed, Lloyd M. Sr
Norelle C. Wildburger, John R. Yates, III, Sung Hwan Yoon, Nicolas L. Young, and Mowei Zhou

ABSTRACT: The Consortium for Top-Down Proteomics (www.topdownproteomics.org) launched the present study to assess the current state of top-down mass spectrometry (TD MS) and middle-down mass spectrometry (MD MS) for characterizing monoclonal antibody (mAb) primary structures, including their modifications. To meet the needs of the rapidly growing therapeutic antibody market, it is important to develop analytical strategies to characterize the heterogeneity of a therapeutic product's primary structure accurately and reproducibly. The major objective of the present study is to determine whether current TD/MD MS technologies and protocols can add value to the more commonly employed bottom-up (BU) approaches with regard to confirming protein integrity, sequencing variable domains, avoiding artifacts, and revealing modifications and their locations. We also aim to gather information on the common TD/MD MS methods and practices in the field. A panel of three mAbs was selected and centrally provided to 20 laboratories worldwide for the analysis: Sigma mAb standard (SiLuLite), NIST mAb standard, and the therapeutic mAb Herceptin (trastuzumab). Various MS instrument platforms and ion dissociation techniques were employed. The present study confirms that TD/MD MS tools are available in laboratories worldwide and provide complementary information to the BU approach that can be crucial for comprehensive mAb characterization. The current limitations, as well as possible solutions to overcome them, are also outlined. A primary limitation revealed by the results of the present study is that the expert knowledge in both experiment and data analysis is indispensable to practice TD/MD MS.

KEYWORDS: *monoclonal antibody, top-down, middle-down, intact mass measurement, mass spectrometry, glycoform*



Optimized workflow for MS quantification of Host Cell Proteins (HCPs) (JPR 2021)

Journal of proteome research

pubs.acs.org/jpr

Article

Optimized Sample Preparation and Data Processing of Data-Independent Acquisition Methods for the Robust Quantification of Trace-Level Host Cell Protein Impurities in Antibody Drug Products

Nicolas Pythoud,[§] Joanna Bons,[§] Geoffroy Mijola, Alain Beck, Sarah Cianférani, and Christine Carapito*

Cite This: https://dx.doi.org/10.1021/acs.jproteome.0c00664

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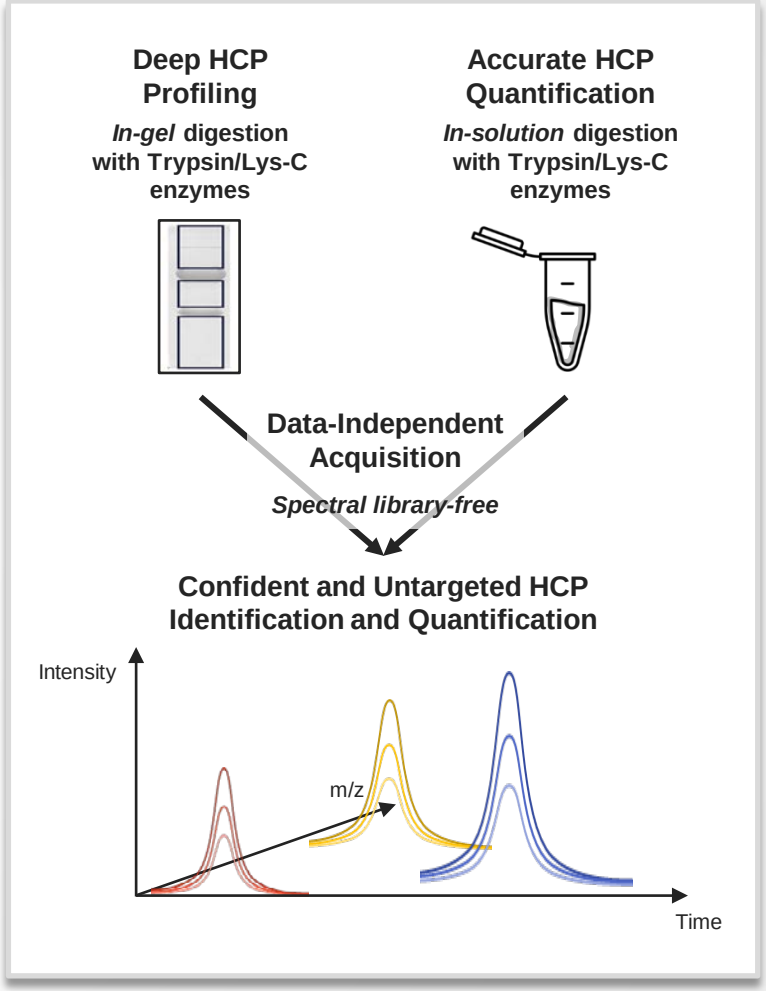


- C. Carapito
- S. Cianférani

Laboratoire de Spectrométrie de Masse Bio-Organique (LSMBO)

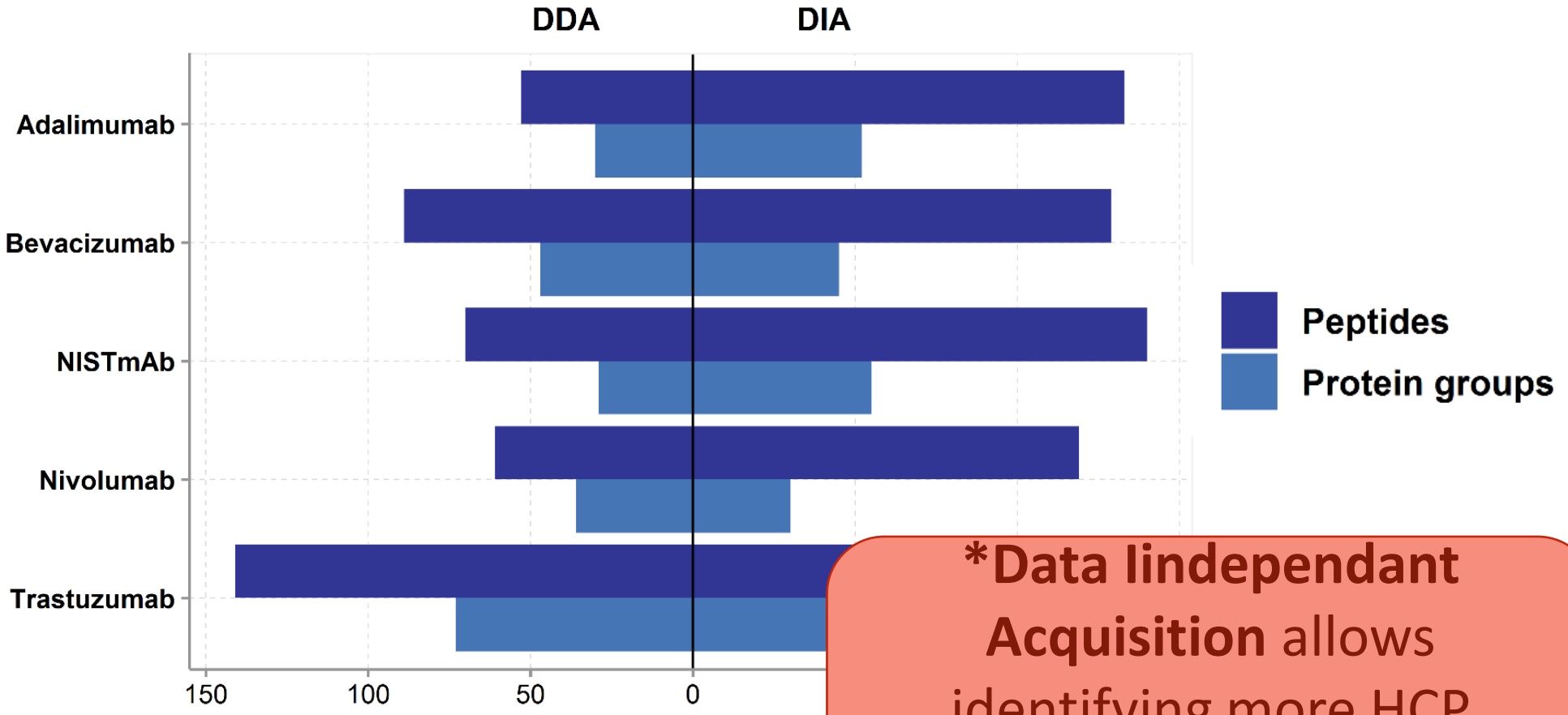
- Adalimumab
- Bevacizumab
- Nivolumab
- Trastuzumab
- NISTmAb

HCP identification in antibody Drug Products

(Data Dependent vs Independant Acquisition)

Identification only

Semi-fractionation



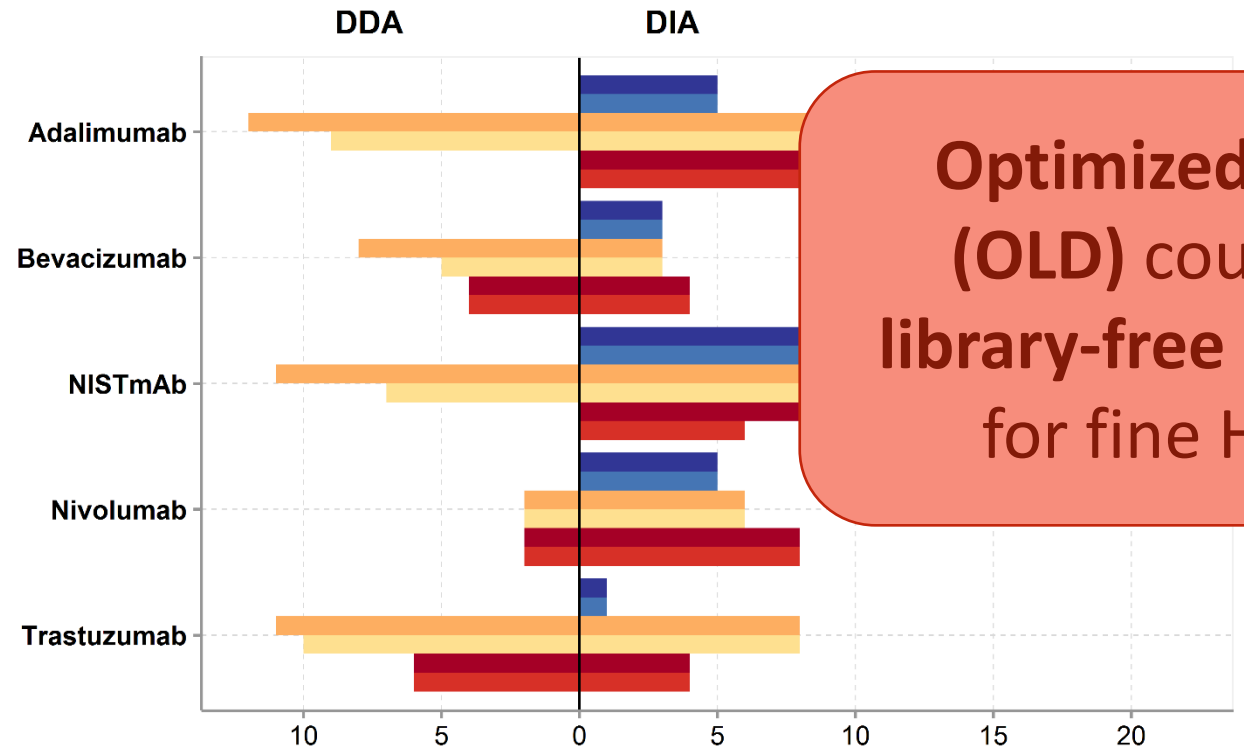
Few 10s of identified HCP

***Data Iindependant Acquisition** allows identifying more HCP
≈ **75%** more **peptides**
≈ **20%** more **protein groups**

- Pythoud N, Bons J, Mijola G, Beck A, Cianferani S, Carapito C, JPR 2020

HCP quantification in antibody Drug Products

Quantification numbers



≈10 quantified HCP

Optimized liquid digestion (OLD) coupled to **spectral library-free DIA** is more suited for fine HCP monitoring

Global HCP amount (ppm)

HCP global amount ≈ 100 ppm

- Pythoud N, Bons J, Mijola G, Beck A, Cianferani S, Carapito C, JPR 2020

MABS
2021, VOL. 13, NO. 1, e1955811 (13 pages)
https://doi.org/10.1080/19420862.2021.1955811

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REPORT

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Host cell protein profiling of commercial therapeutic protein drugs as a benchmark for monoclonal antibody-based therapeutic protein development

Rosalynn Molden[a], Mengqi Hu[a], Sook Yen E[a], Diana Saggese[a], James Reilly[b], John Mattila[b], Haibo Qiu[a], Gang Chen[c], Hanne Bak[b], and Ning Li[a]

[a]Analytical Chemistry, Regeneron Pharmaceuticals, Inc, Tarrytown, New York, USA; [b]Preclinical Manufacturing and Process Development, Regeneron Pharmaceuticals, Inc, Tarrytown, New York, USA; [c]Protein Expression Sciences, Regeneron Pharmaceuticals, Inc, Tarrytown, New York, USA

ABSTRACT

Therapeutic proteins including monoclonal antibodies (mAbs) are usually produced in engineered host cell lines that also produce thousands of endogenous proteins at varying levels. A critical aspect of the development of biotherapeutics manufacturing processes is the removal of these host cell proteins (HCP) to appropriate levels in order to minimize risk to patient safety and drug efficacy. During the development process and associated analytical characterization, mass spectrometry (MS) has become an increasingly popular tool for HCP analysis due to its ability to provide both relative abundance and identity of individual HCP and because the method does not rely on polyclonal antibodies, which are used in enzyme-linked immunosorbent assays. In this study, HCP from 29 commercially marketed mAb and mAb-based therapeutics were profiled using liquid chromatography (LC)-MS/MS with the identification and relative quantification of 79 individual HCP in total. Excluding an outlier drug, the relative levels of individual HCP determined in the approved therapeutics were generally low, with an average of 20 ppm (µmol HCP/mol drug) measured by LC-MS/MS, and only a few (<7 in average) HCP were identified in each drug analyzed. From this analysis, we also gained knowledge about which HCP are frequently identified in mAb-based products and their typical levels relative to the drugs for the identified individual HCP. In addition, we examined HCP composition from antibodies produced in house and found our current development process brings HCP to levels that are consistent with marketed drugs. Finally, we described a specific case to demonstrate how the HCP information from commercially marketed drugs could inform future HCP analyses.

mAbs

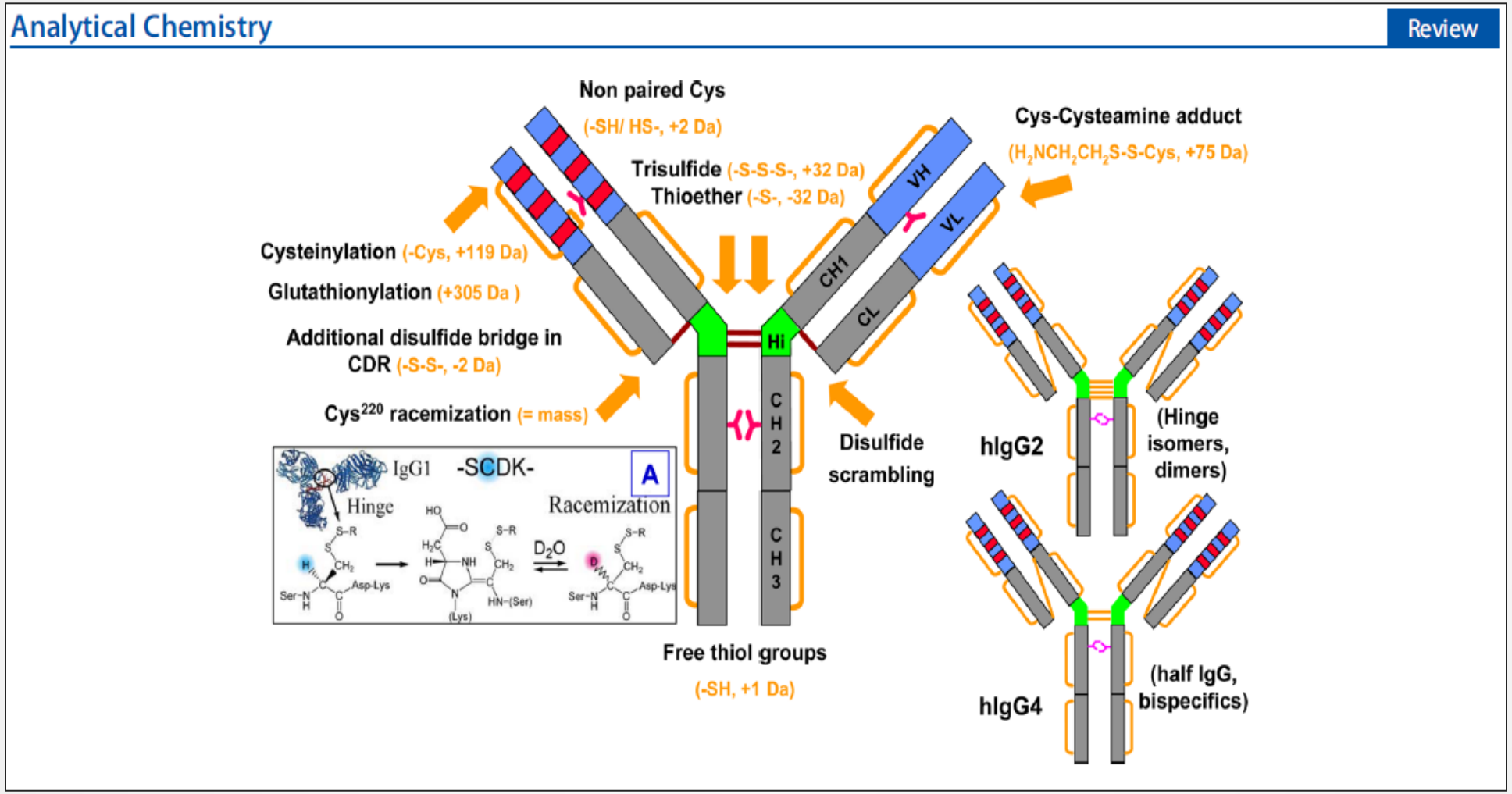
- 29 FDA/ EMA approved antibody based products benchmarks
- LC-MS/ MS: 79 individual HCPs
- 20 ppm

(2) mAbs structure & Critical Quality Attributes (CQAs)

- Cys-related, Size, Oxidized, Glyco & Charged variants assessment

Pierre Fabre

(2.1) IgGs: Cys-linked variants (Isotype, -CysSH-…)



➢ **Beck A, Wagner E, Ayoub D, Van Dorsselaer A, Cianferani S, Anal Chem 2013**

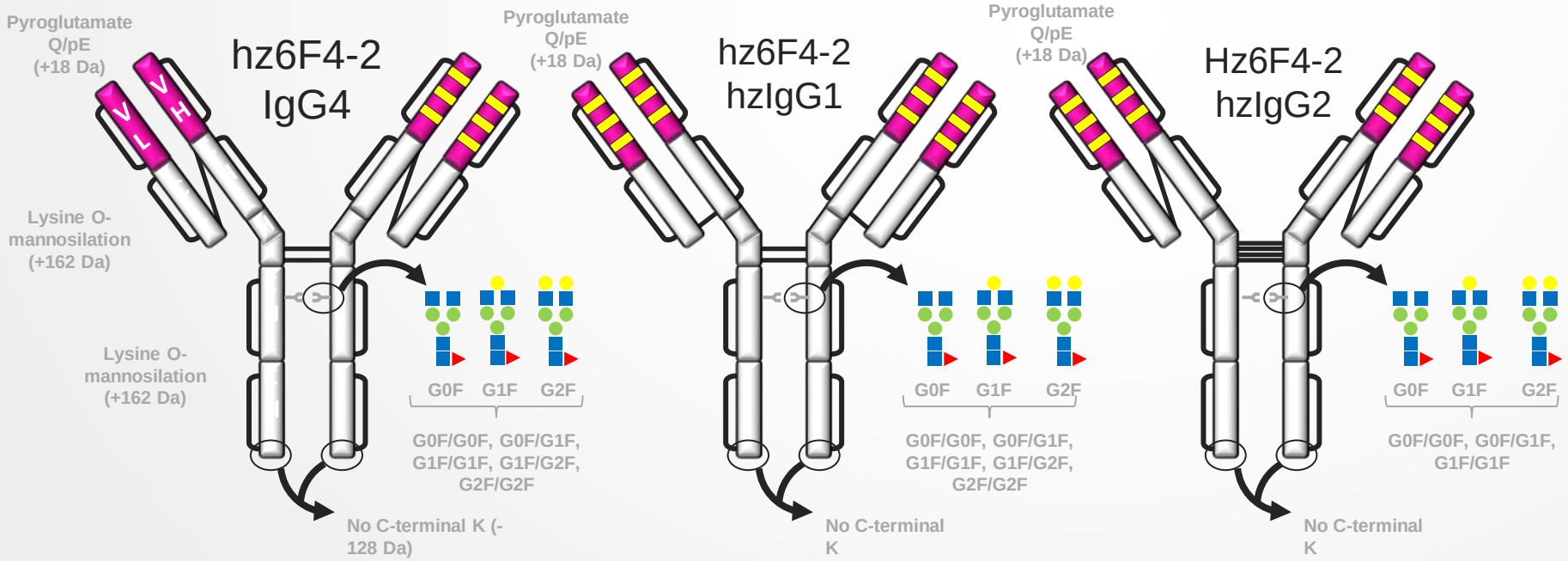
HzIgG1, 2 & 4 subtypes (Structural Bio 2020)

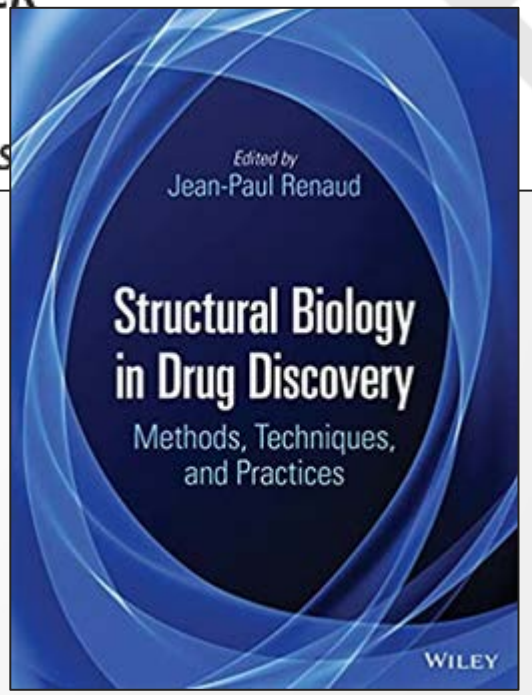
Mass Spectrometry-Based Strategies for Therapeutic Antibodies Extensive Characterization and Optimization (OptimAbs)

Amandine Boeuf[1], François Debaene[2], Daniel Ayoub[1], Hélène Diemer[2], Anthony Ehkirch[2], Elsa Wagner-Rousset[1], Alain Van Dorsselaer[2], Sarah Cianférani[2], and Alain Beck[1]

[1] *Centre d'Immunologie Pierre-Fabre, Saint-Julien en Genevois, France*

[2] *Laboratoire de Spectrométrie de Masse BioOrganique, Institut Pluridisciplinaire Hubert Curien, Université de Strasbourg, CNRS*





Middle-level-IM-MS & CIU (Anal Chem 2020)

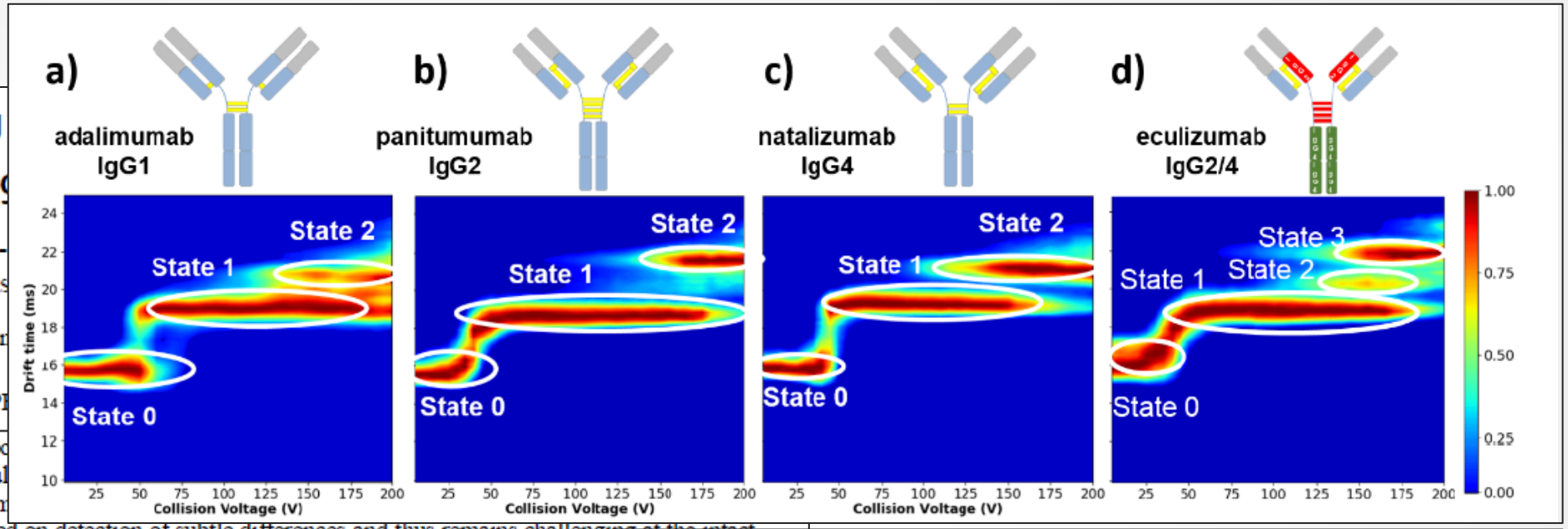
Middle-level IM-MS and CIU immunoglobulin isotype fing

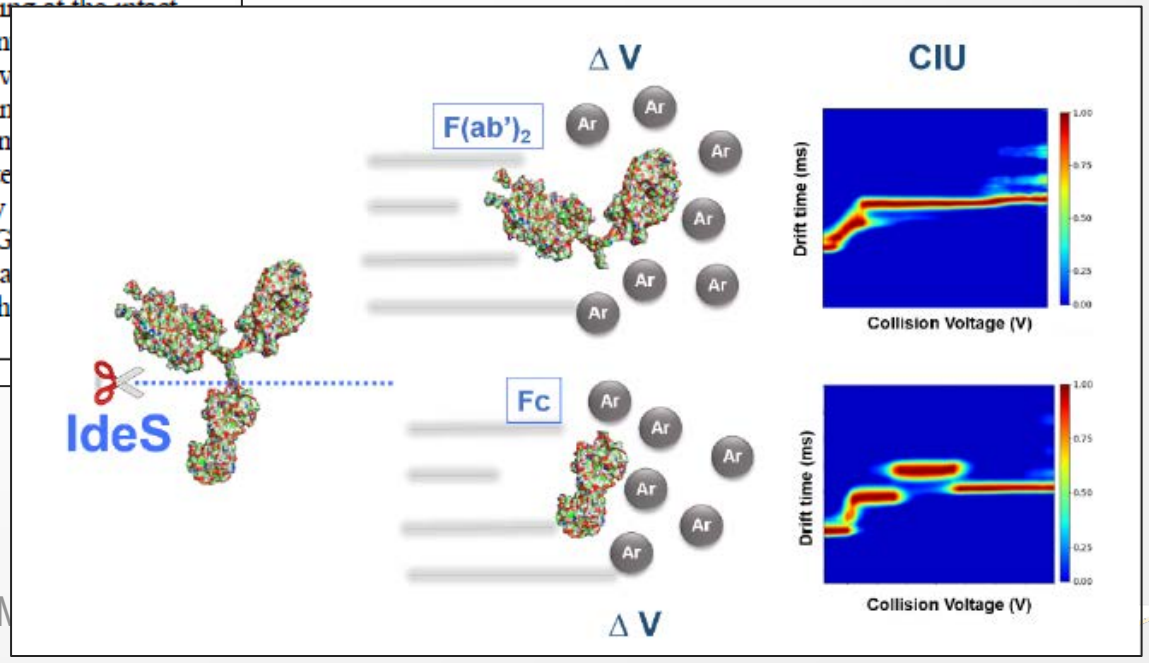
Thomas Botzanowski[1¶], Oscar Hernandez-Desligni[1], Olivier Colas[2], Jean-François

1 Laboratoire de Spectrométrie de Masse BioOrgan bourg, France.

2 IRPF - Centre d'Immunologie Pierre-Fabre (CIP

ABSTRACT: Currently approved therapeutic monoc types, which differ in their specific inter-chains disul for mAb isotyping, among which native ion mobility m However, mAb isotyping by these approaches is based on detection of subtle differences and thus remains challengi level. We report here on middle-level (after IdeS digestion) IM-MS and CIU approaches to afford better differen isotypes. Our method provides simultaneously CIU patterns of F(ab')$_2$ and Fc domains within a single run. Middle-lev of F(ab')2 domains enable more reliable classification of mAb isotypes compared to intact level CIU, while CIU fin domains are overall less informative for mAb isotyping. F(ab')$_2$ regions can thus be considered as diagnostic don specific CIU signatures for mAb isotyping. Benefits of middle-level IM-MS and CIU approaches are further illustrate IgG2/IgG4 eculizumab. While classical analytical techniques led to controversial results, middle-level CIU uniquely the challenge of eculizumab « hybridicity », highlighting that its F(ab')$_2$ and Fc CIU patterns corresponds to an IgG respectively. Altogether, the middle-level CIU approach is more clear-cut, accurate and straightforward for canonica complex, engineered next generation mAb formats isotyping. Middle-level CIU thus constitutes a real breakthrough protein analysis, paving the way for its implementation in R&D laboratories.





- **O. Hernandez-Alba, S. Cianférani**

SEC-CIU: workflow automation (Anal Chem 2020)

Analytical Chemistry

Towards automation of Collision Induced Unfo through online Size Exclusion Chromatography Mass Spectrometry.

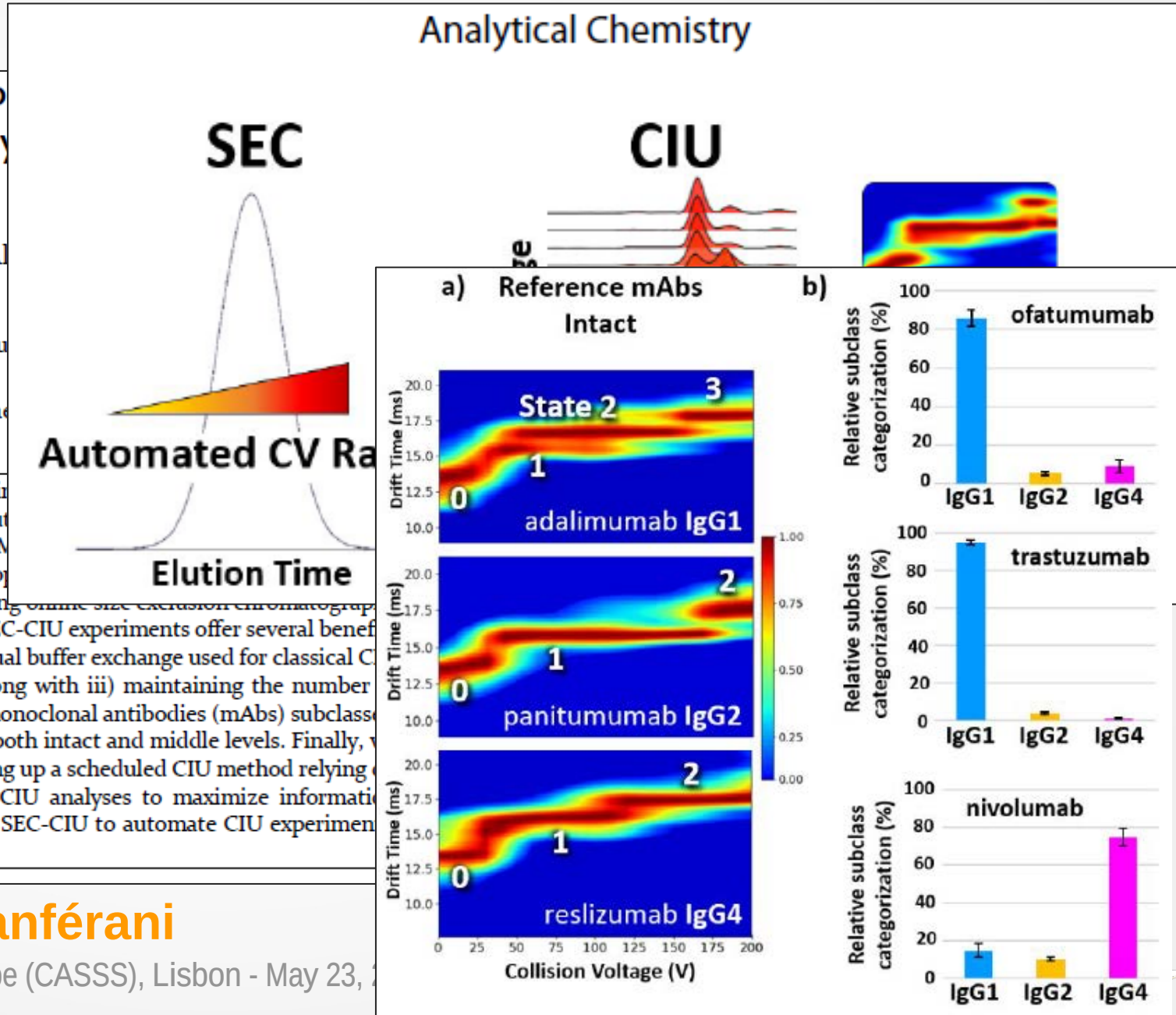
Evolène Deslignière[1], Anthony Ehkirch[1], Thomas Botzanowski[1], Al Alba[1], Sarah Cianférani[1*]

[1] Laboratoire de Spectrométrie de Masse BioOrganique, Université de Strasbou Strasbourg, France.

[2] IRPF - Centre d'Immunologie Pierre-Fabre (CIPF), 74160 Saint-Julien-en-Gene

*Corresponding author: Sarah Cianférani. Email: sarah.cianferani@unistra.fr

ABSTRACT: Ion mobility-based collision induced unfolding (CIU) has gained in unfolding of proteins and their noncovalent complexes, notably for biotherapeut changes of proteins and emerges as an attractive alternative to circumvent poor IM automation for buffer exchange and data acquisition, precluding its wide adop workflow for CIU experiments, from sample preparation to data interpretation using coupled to native ion mobility mass spectometry (SEC-CIU). Online automated SEC-CIU experiments offer several benef over nanoESI-CIU, among which i) improved and fast desalting compared to manual buffer exchange used for classical C experiments; ii) drastic reduction of the overall data collection time process along with iii) maintaining the number unfolding transitions. We then evaluate the potential of SEC-CIU to distinguish monoclonal antibodies (mAbs) subclass illustrating the efficiency of our method for rapid mAb subclass identification at both intact and middle levels. Finally, demonstrate that CIU data acquisition time can be further reduced either by setting up a scheduled CIU method relying diagnostic trap collision voltages or by implementing mAbs-multiplexed SEC-CIU analyses to maximize informati content in a single experiment. Altogether, our results confirm the suitability of SEC-CIU to automate CIU experimen particularly for the fast characterization of next generation mAb-based products.



- **E. Desligniere, S. Cianférani**

CE-SDS: 26 mAbs + 2 ADCs FDA approv. (JPBA 2020)

Journal of Pharmaceutical and Biomedical Analysis 184 (2020) 113166

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Determination of size variants by CE-SDS for approved therapeutic antibodies: Key implications of subclasses and light chain specificiti

Elsa Wagner[a], Olivier Colas[a], Stéphane Chenu[a], Alexandre Goyon[b], Amarande Murisie
Sarah Cianferani[c], Yannis François[d], Szabolcs Fekete[b], Davy Guillarme[b],
Valentina D'Atri[b,*], Alain Beck[a,*]

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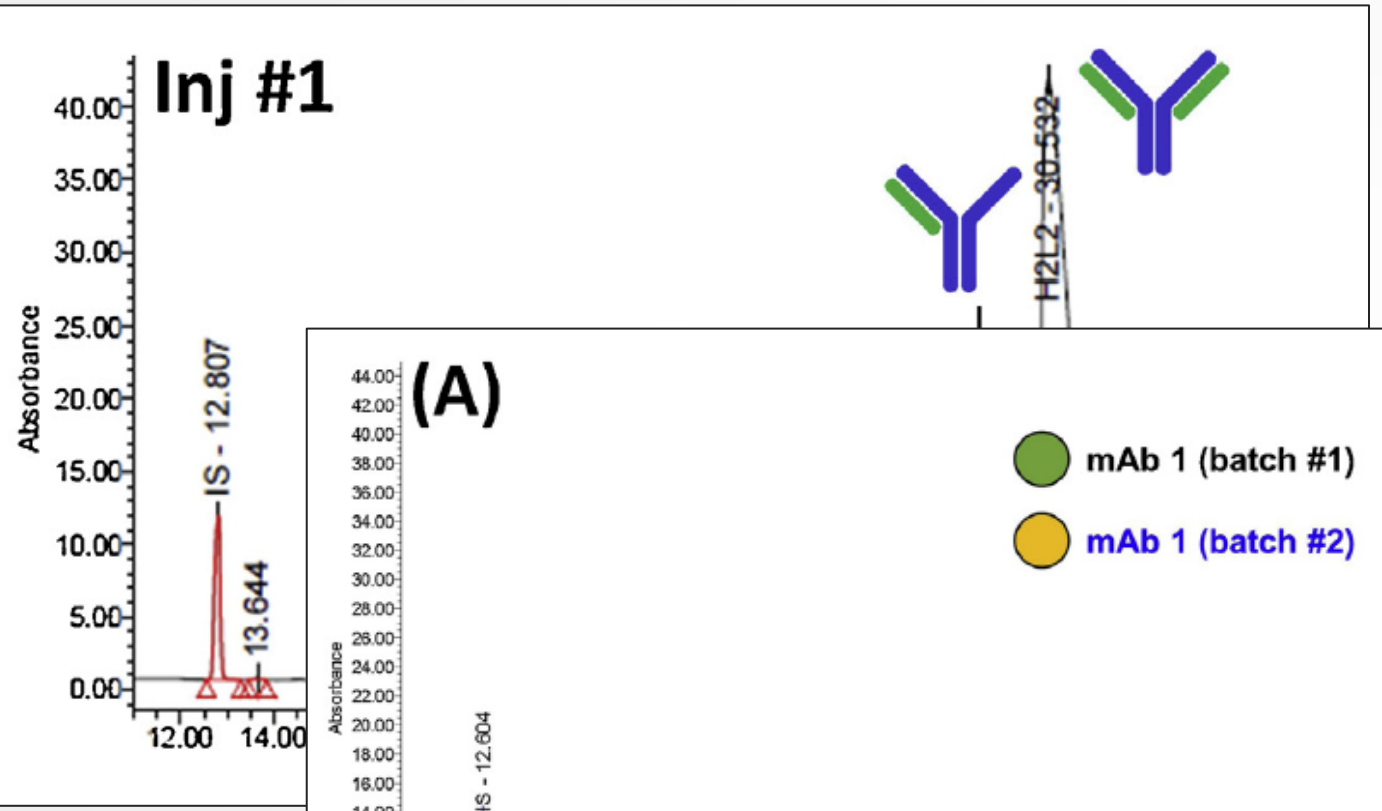
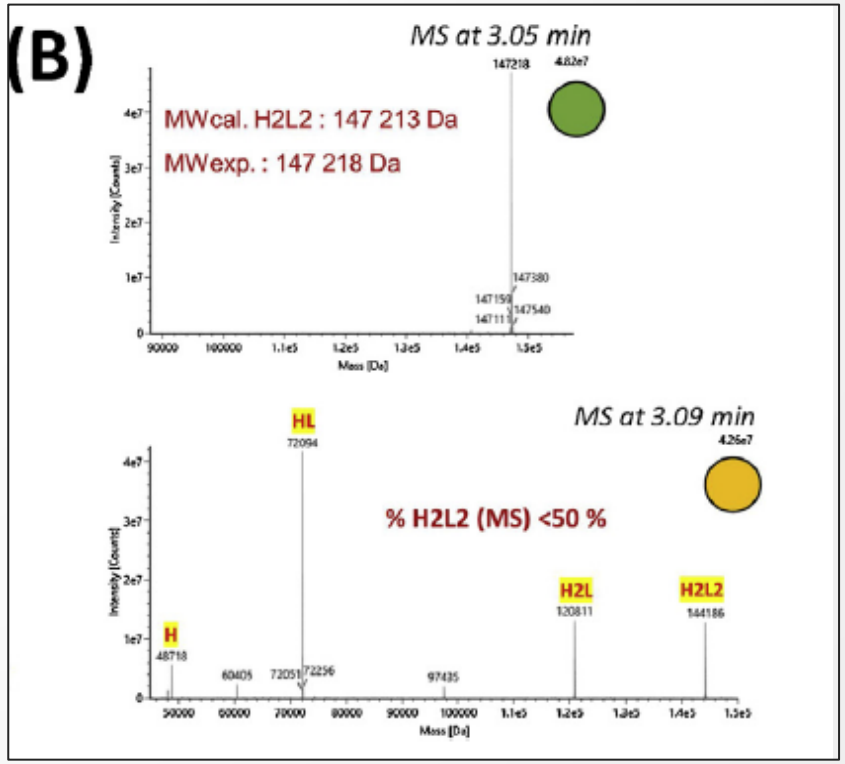
[b] Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, CMU-Rue Michel Servet 1, 1211 Geneva 4, Switzerland
[c] Laboratoire de Spectrométrie de Masse BioOrganique, IPHC UMR 7178, Université de Strasbourg, CNRS, Strasbourg, France
[d] Laboratoire de Spectrométrie de Masse des Interactions et des Systèmes (LSMIS), UMR 7140, Université de Strasbourg, CNRS, Strasbourg, France

- Ch, Hz, Hu IgGs
- CHO, NS0, SP2/0
- IgG1, 2, 2/4, 4wt, 4stab
- Glyco-engineered
- A-glycosylated
- Kappa & lambda LC
- Partially reduced IgGs
- Biosimilars
- Hinge Cys & Lys ADCs
- NISTmab

nrCE-SDS IgG profiles: partially reduced profiles

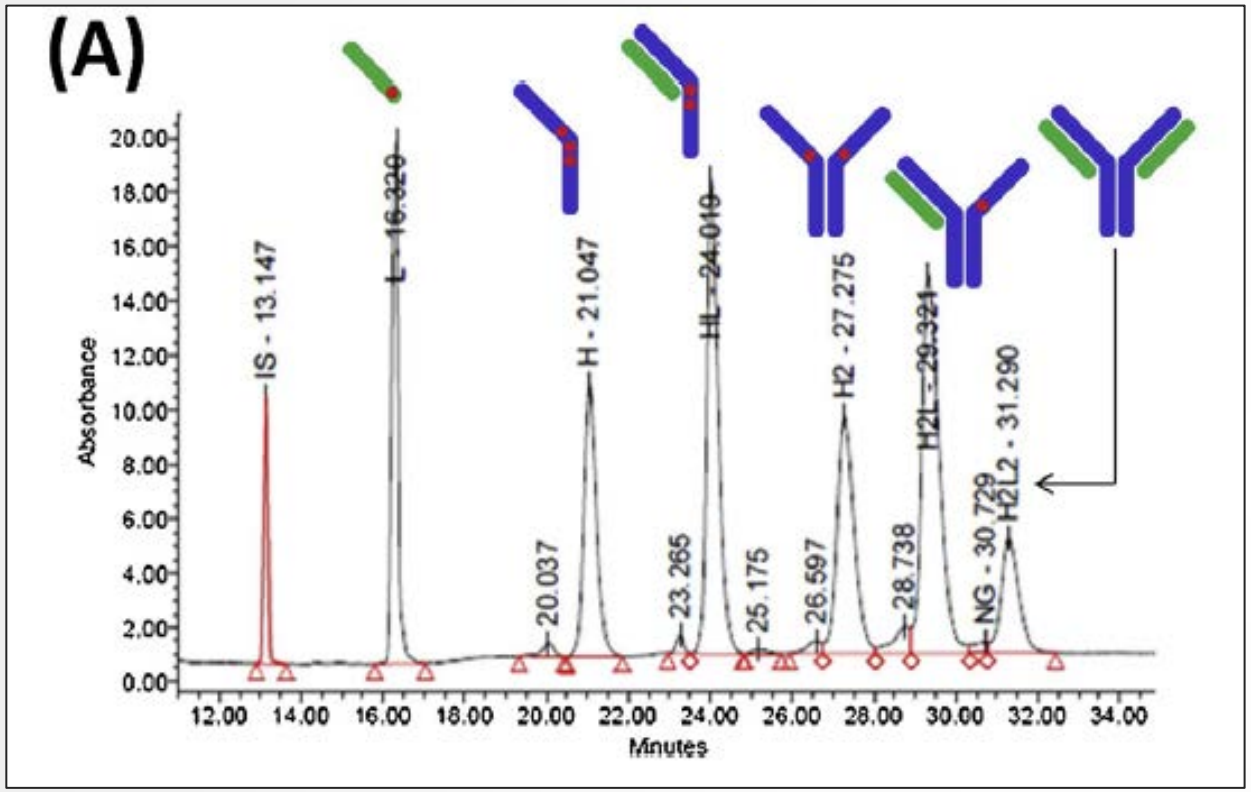


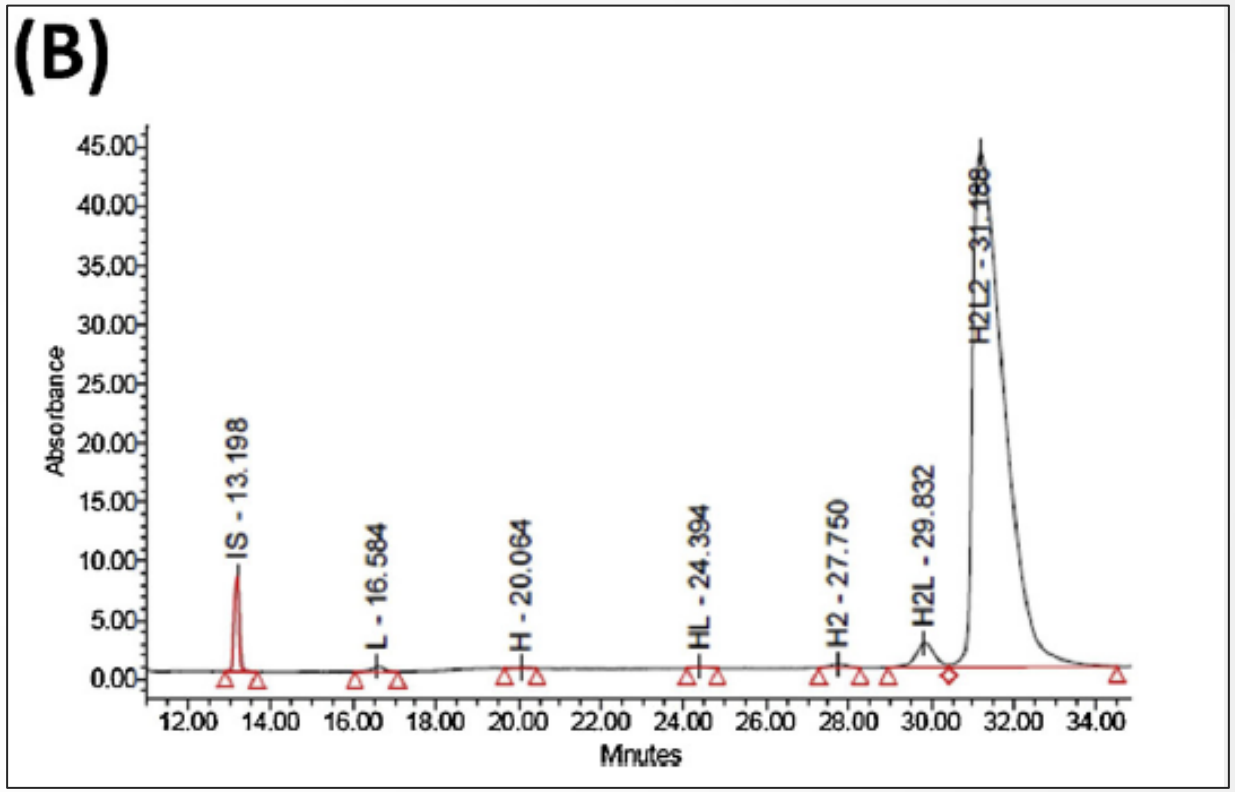
- **nrCE-SDS partially reduced IgG profile (denaturing conditions)**
- **“H2L2” in native conditions (SEC, CEX…)**
- **Functionally active (ELISA, FcgR, FcRn…)**



- **Wagner E, Beck A et al, JPBA 2020**

nrCE-SDS profiles: Adcetris vs Kadcyla



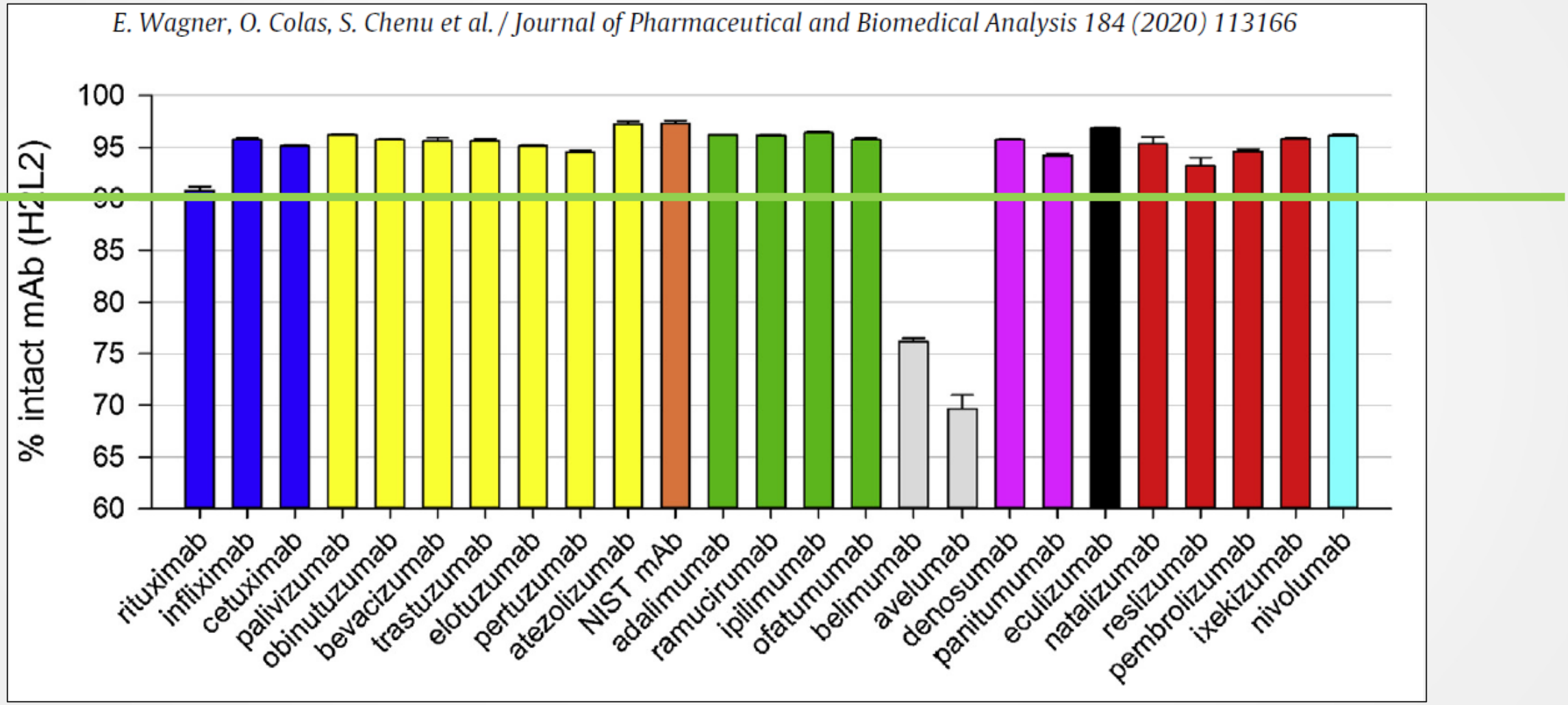


- Adcetris: Hinge LCys-conjugated ADC,
- nr CE-SDS “partially reduced profile”
- “H2L2” in native conditions (SEC, native MS)
- “ Functional” test OK (ELISA, FcgR, FcRn…)

- Kadcyla: Lys-conjugated ADC
- nrCE-SDS comparable to trastuzumab

- Wagner E, Beck A et al, JPBA 2020

nrCE-SDS profiles (H2L2): FDA approved IgGs



- **Standard nrCE-SDS conditions: H2L2 > 90/95% excepted for belimumab and avelumab**
- **Wagner E, Beck A et al, JPBA 2020**

nrCE-SDS : hIg1Gkappa vs lambda

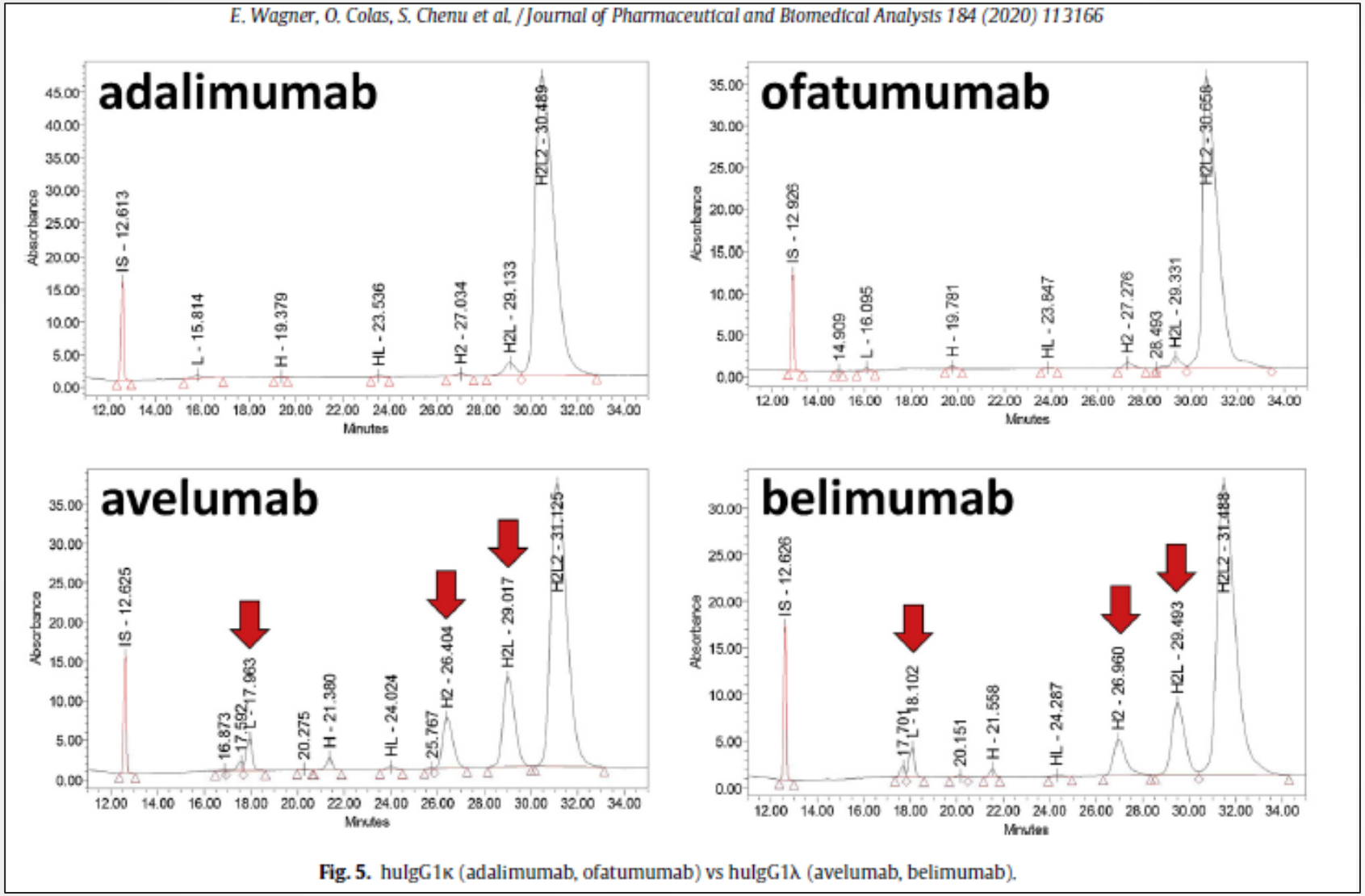


Fig. 5. hulgG1κ (adalimumab, ofatumumab) vs hulgG1λ (avelumab, belimumab).

- **Terminal Ser in lamda light chains have a significant impact on the stability of the interchain L-H binding**
- **This bond weaker than the disulfide bond of the interchain H-H binding**
- **Application of generic nrCE-SDS method for the analysis of huIgG1 lambda may be biased by the sample preparation step (denaturation step at 70◦C for 10 min)**
- **Require further optimization to be successfully applied to this peculiar subclass**

- **Wagner E, Beck A et al, JPBA 2020**

HR-IMMS for disulfide bridge pairing in CDRs (JASMS 2021)

ACS Partner Journal

Journal of the American Society for Mass Spectrometry

pubs.acs.org/jasms

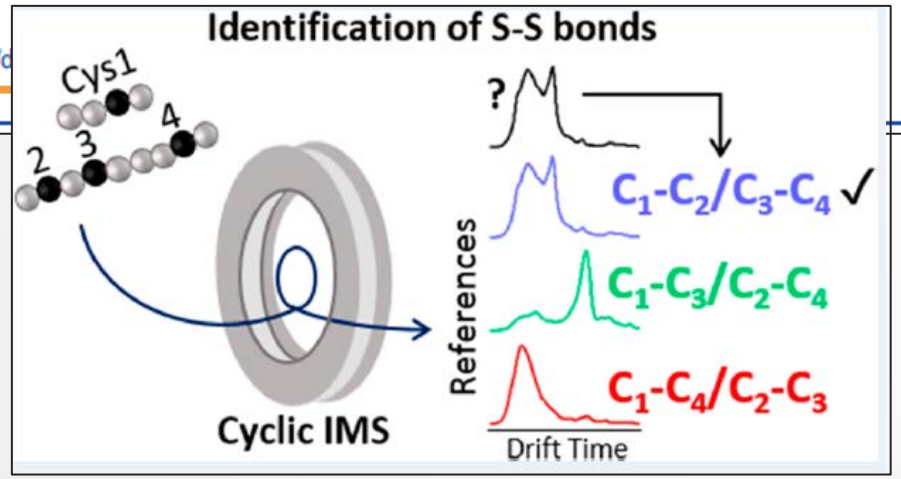
Research Article

Laboratoire de Spectrométrie de LSMBO Masse Bio-Organique

High-Resolution IMS–MS to Assign Additional Disulfide Bridge Pairing in Complementarity-Determining Regions of an IgG4 Monoclonal Antibody

Evolène Deslignière,[§] Thomas Botzanowski,[§] Hélène Diemer, Dale A. Cooper-Shepherd, Elsa Wagner-Rousset, Olivier Colas, Guillaume Béchade, Kevin Giles, Oscar Hernandez-Alba, Alain [illegible] and Sarah Cianférani*

Cite This: https://d



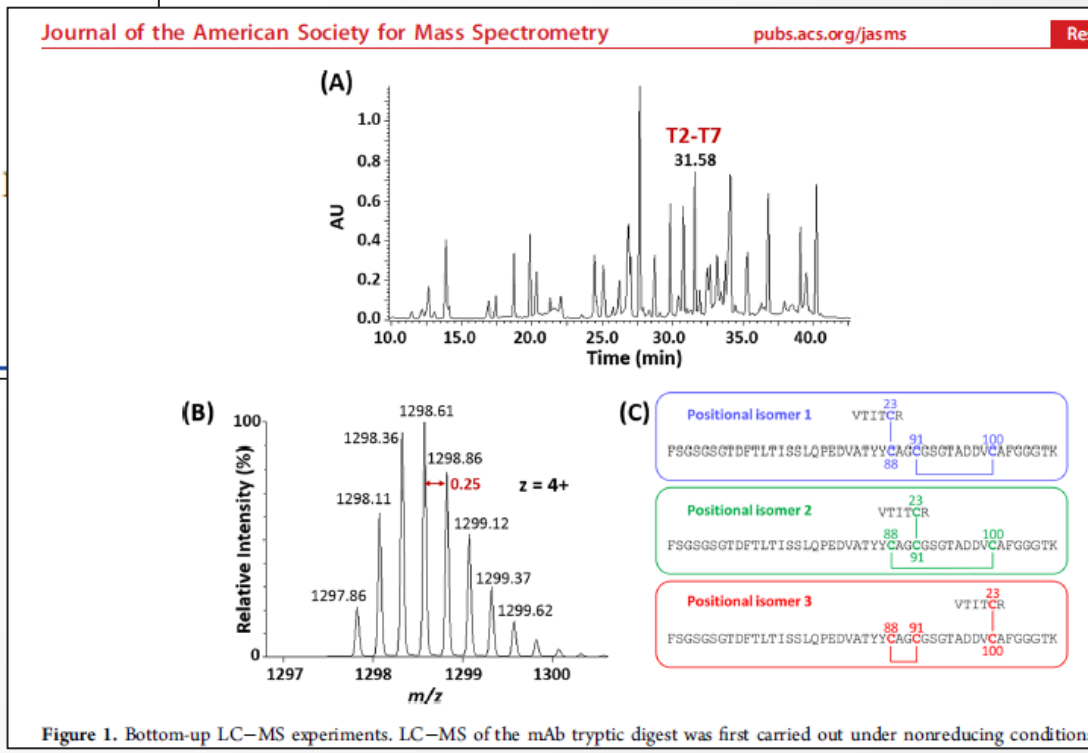
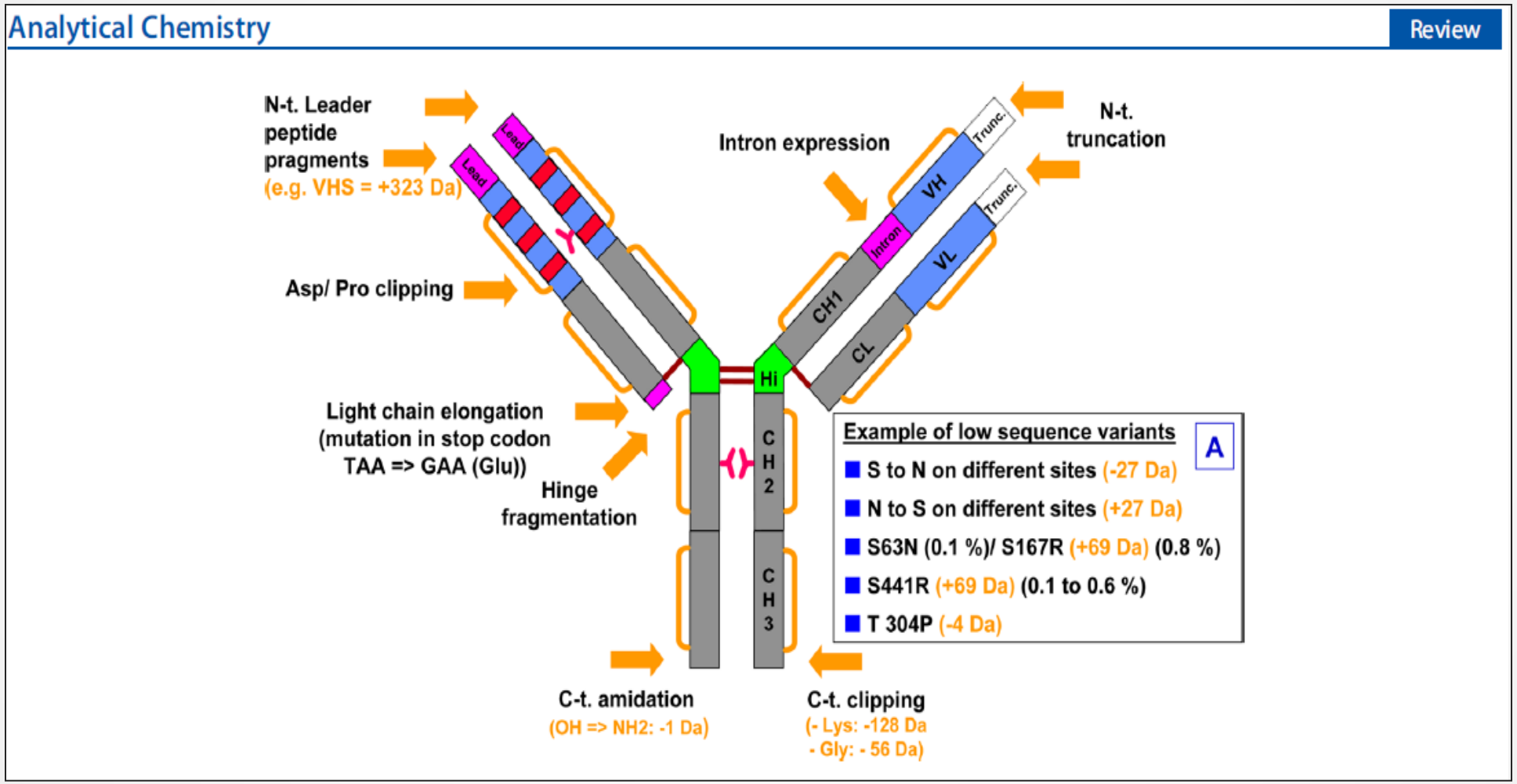


Figure 1. Bottom-up LC–MS experiments. LC–MS of the mAb tryptic digest was first carried out under nonreducing condition

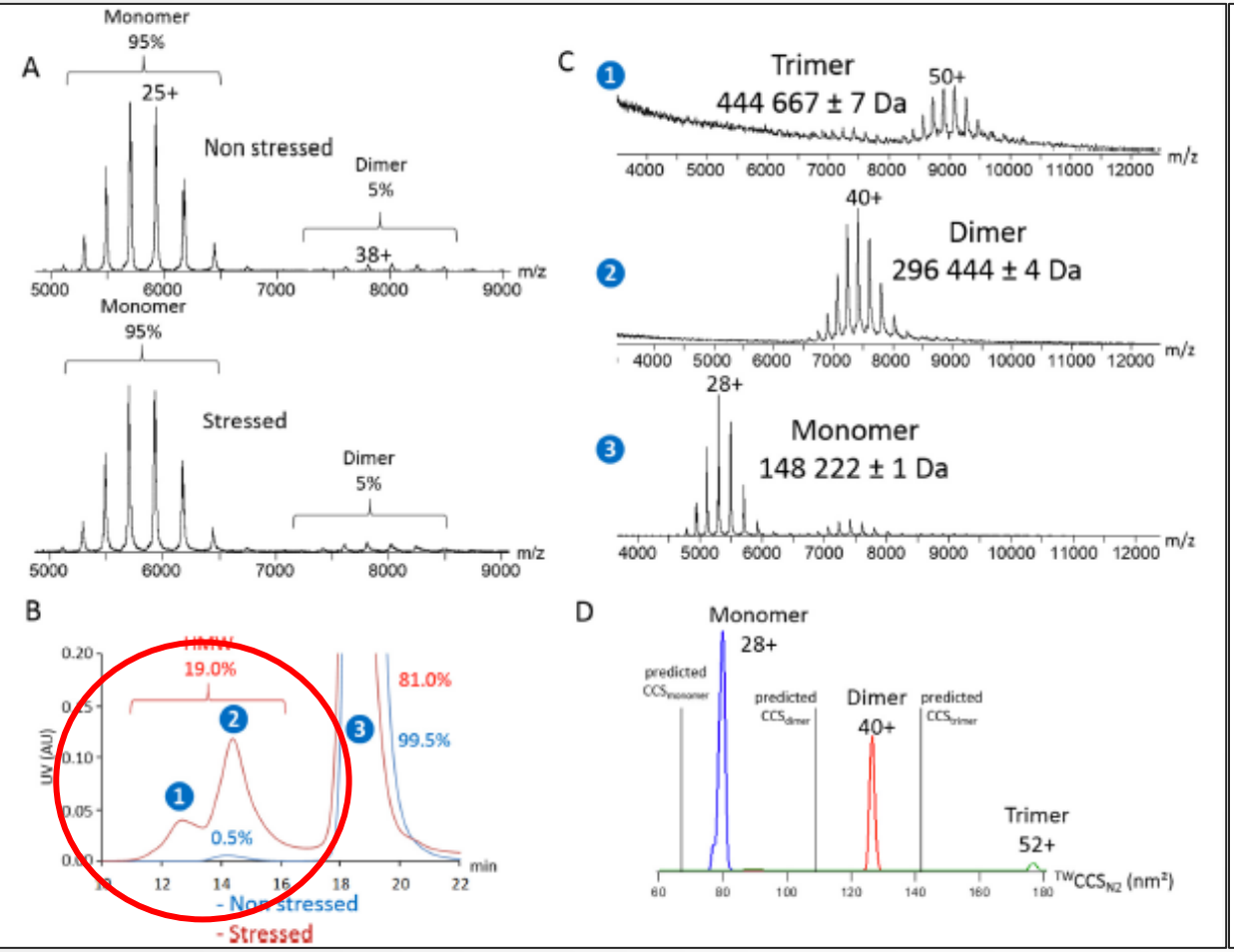
(2.2) IgGs size variants (N-Top/ Extented Bottom-Up)



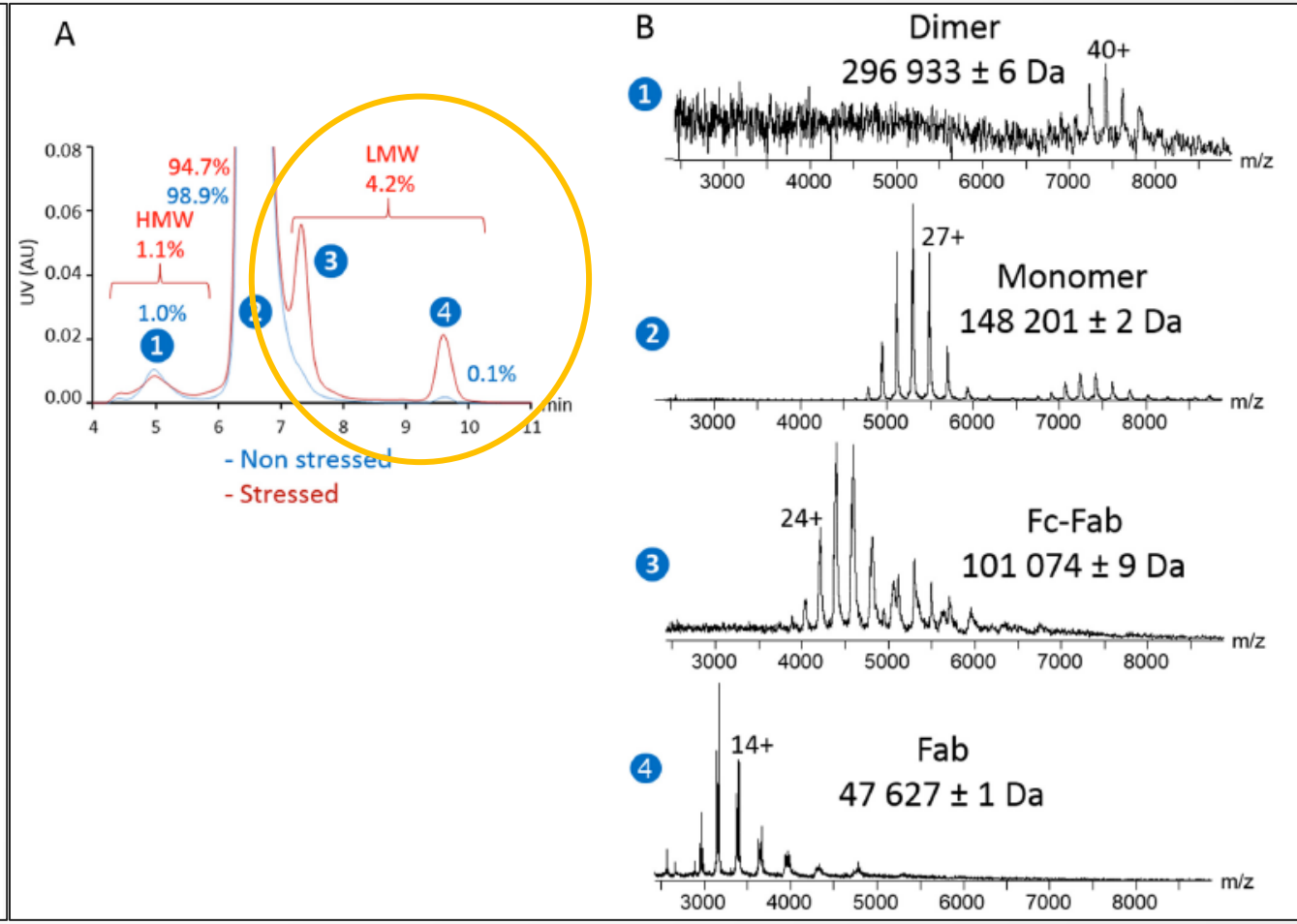
- Beck A, Wagner E, Ayoub D, Van Dorsselaer A, Cianferani S, Anal Chem 2013
- Fekete S, Beck A, Veuthey JL, Guillarme D, JPBA 2014 (SEC)
- Ayoub D, Bertaccini D, Beck A, Schaeffer-Reiss C et al, Anal Chem 2015

Size variants structure assessement: SEC-native MS

(A) Monomer & HMWS (trastuzumab)



(B) Monomer & LMWS (NISTmAb)

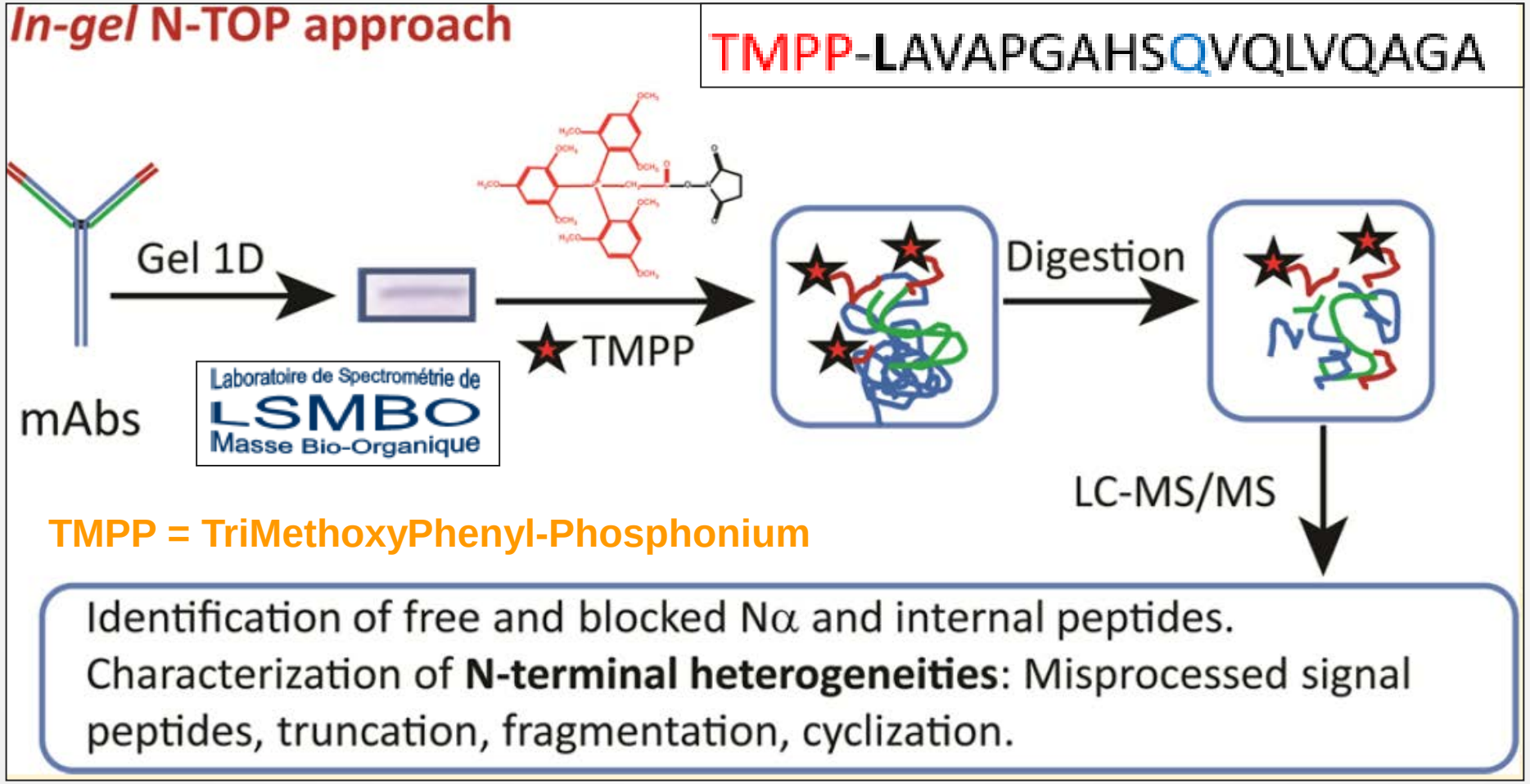


A. Ehkirch & coll.

- Ehkirch A, Beck A, Cianferani S et al, J Chrom B 2018

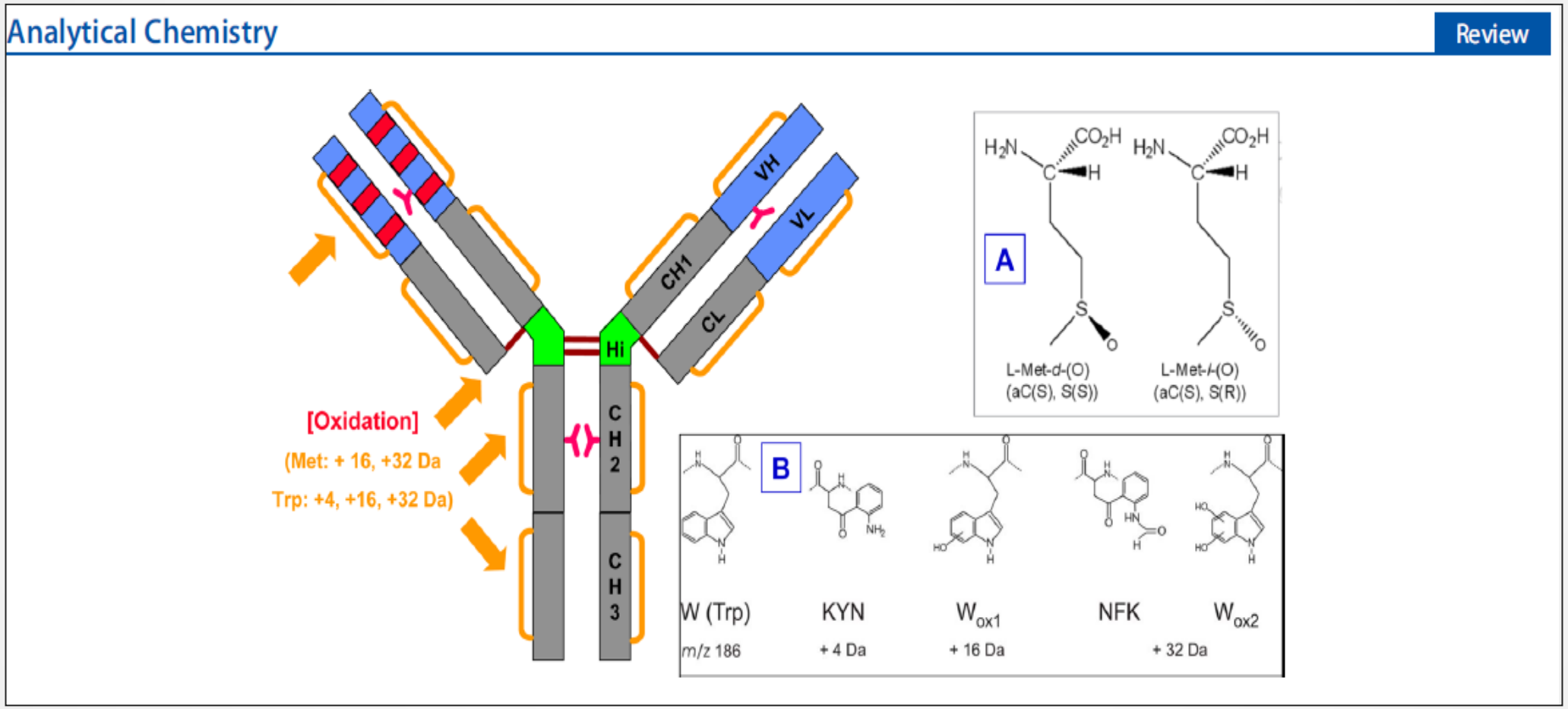
Natalizumab: N-t oriented proteomics (N-Top, bottom)

N-Terminal in-gel charge derivatization of α-Amines + LC-MS/MS



➢ **Ayoub D, Bertaccini D, Beck A, Cianferani S, Schaeffer-Reiss C et al, Anal Chem 2015**

(2.3) IgGs: oxidized variants (Met, Trp)



- Beck A, Wagner E, Ayoub D, Van Dorsselaer A, Cianferani S, Anal Chem 2013
- Regl C, Wohlschlager T, Holzmann J, Huber CG, Anal Chem 2017

On line 4D for mAbs size variants (SECxSEC-IMxMS)

Analytical Chemistry Article

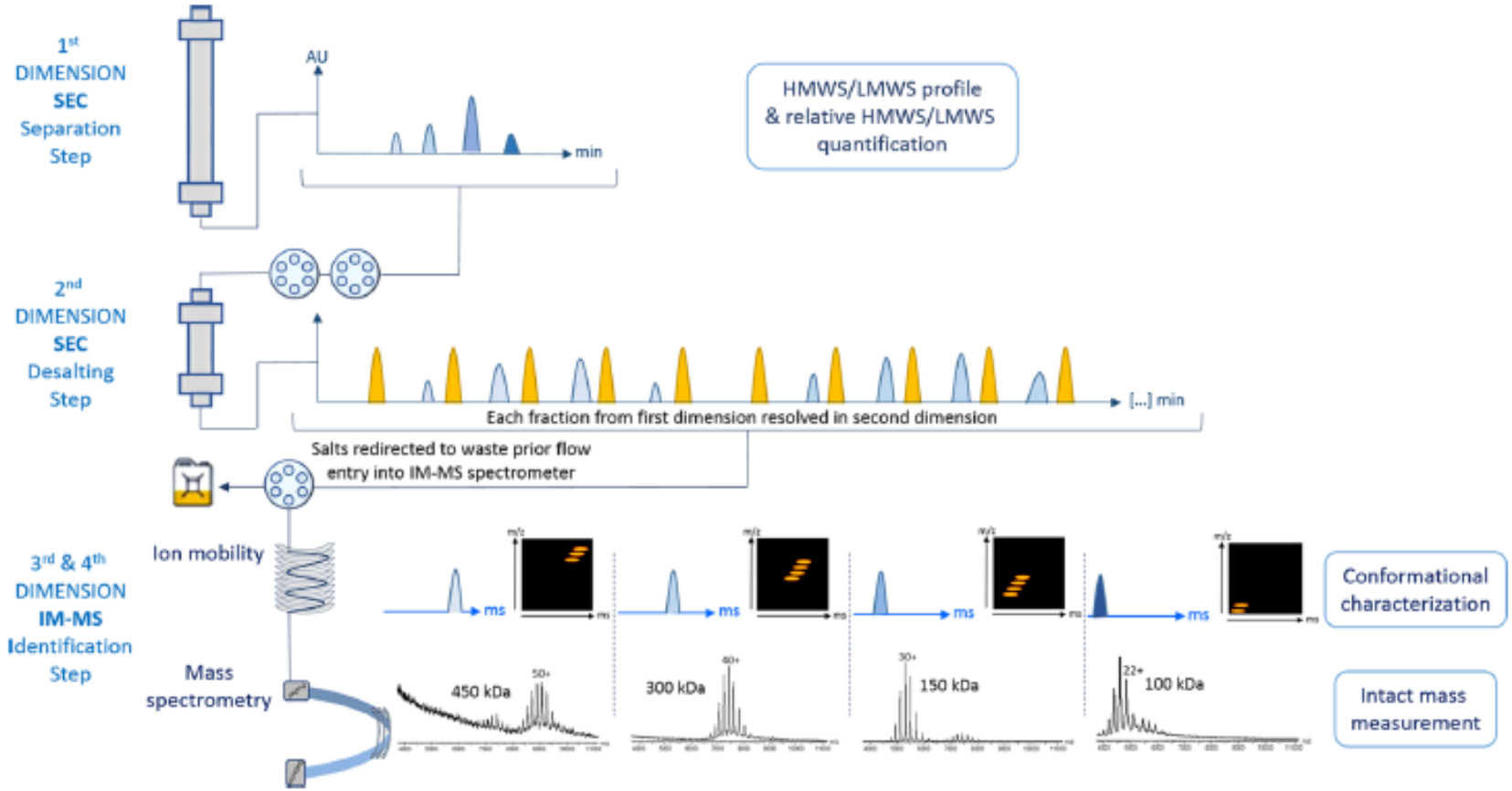
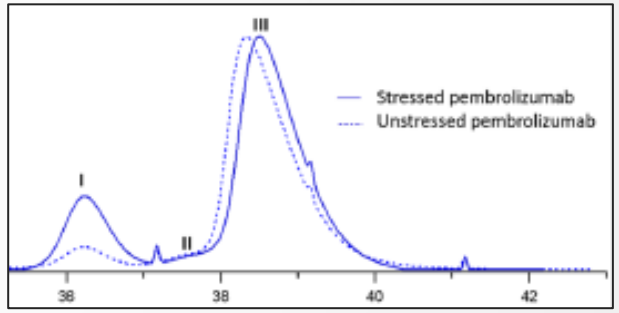


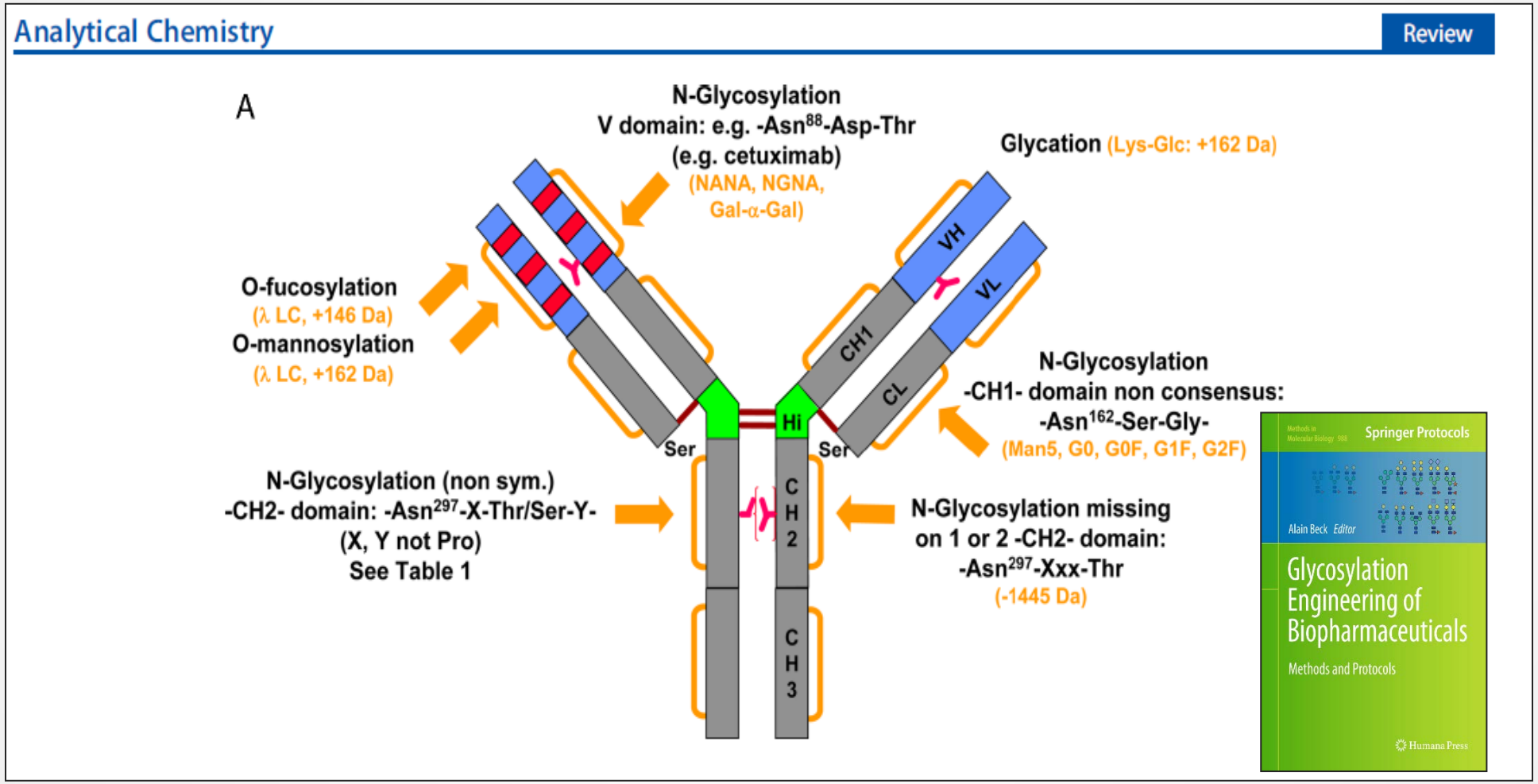
Figure 2. Flowchart of the SEC×SEC-native IM×MS for mAb analysis. The optimized SEC×SEC method was hyphenated to IM-MS. In the first dimension, SEC with nonvolatile salts allows a proper separation and quantitation of mAb HMWS/LMWS. In the second dimension, a short SEC column used with a volatile mobile phase was employed as a fast desalting step. Online native IM-MS allows conformational characterization and intact mass measurement of each individual ^{1}D-SEC peaks.



Pembrolizumab HMWS (SEC): oxidized species and not dimers or aggregates

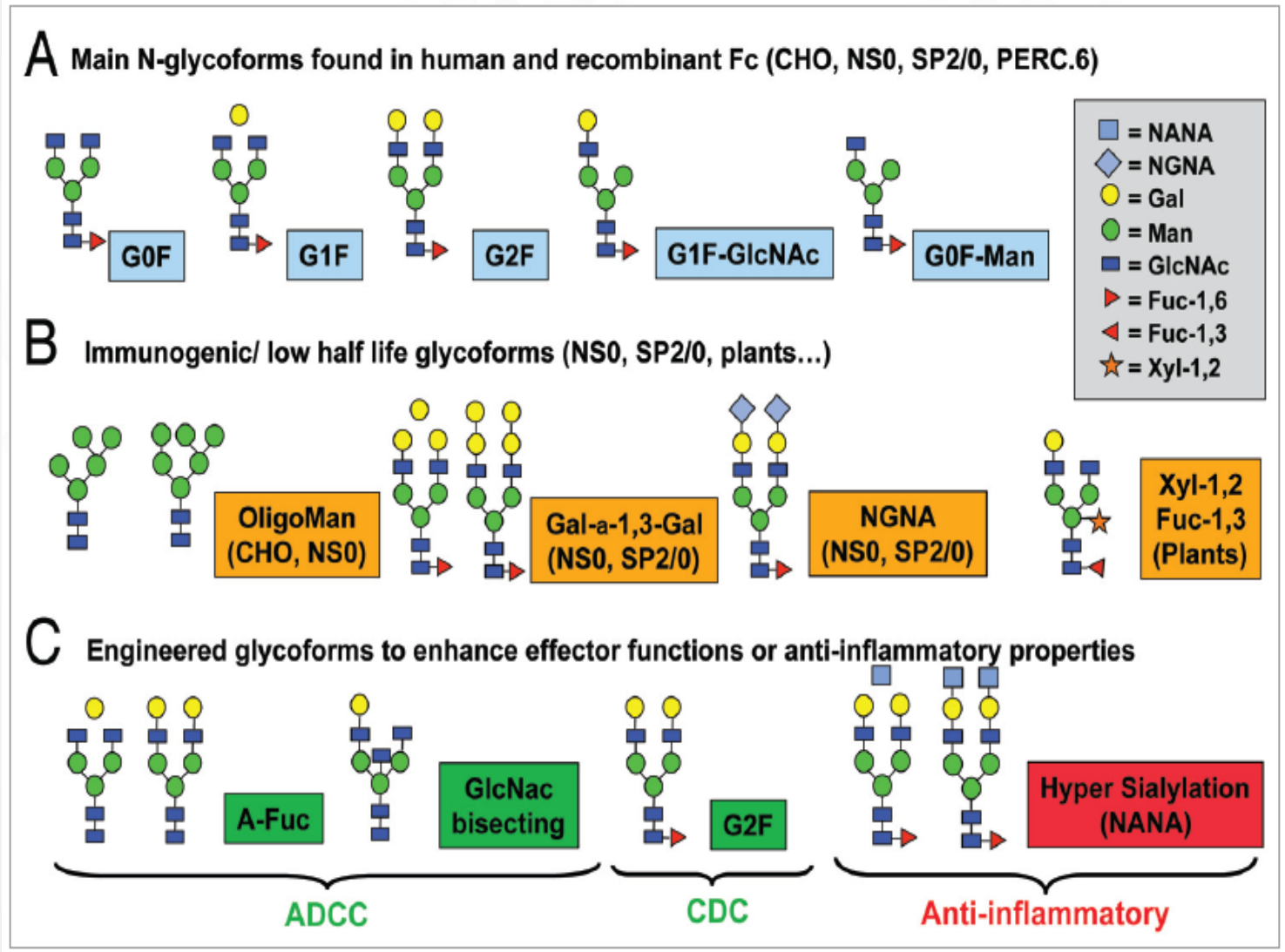
- **Ehkirch A, D'Atri V, Rouvière F, Beck A, Guillarme D, Heinisch S, Cianférani S et al. Anal Chem 2018**

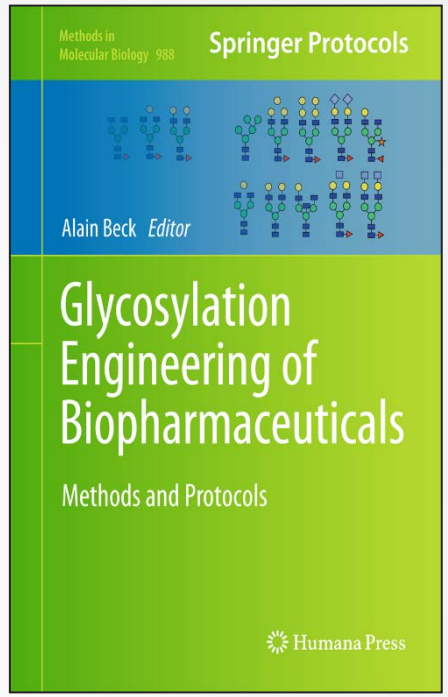
(2.4) IgGs/ADCs/Fc-fusions: Glycovariants (N- & O-)



- Beck A, Wagner E, Ayoub D, Van Dorsselaer A, Cianferani S, Anal Chem 2013
- Beck A , Methods Mol Biol 2013 - Beck A et al, J Mass Spec 2015
- National Institute of Standards and Technology (NIST) International Study (GlycoNISTmAb)

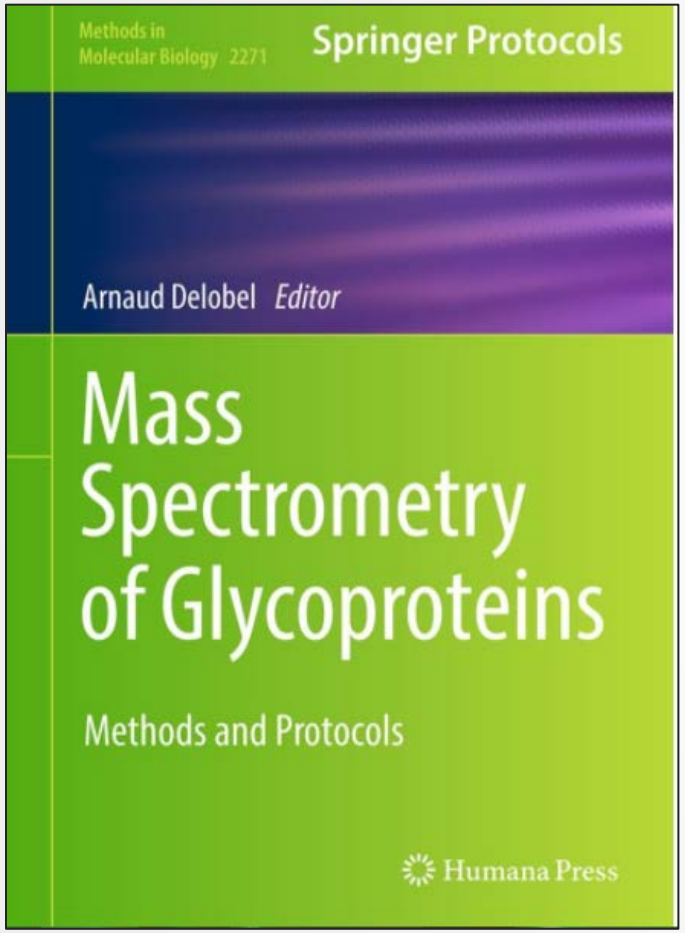
mAbs N-glycovariants: Critical Quality Attributes





- Beck A et al, Curr Pharm Biotech 2008
- Beck A & Reichert JM, mAbs 2012
- Beck A et al, Anal Chem 2013
- Beck A, Meth Mol Biol 988, 2013

MS of Glycoproteins, M&P, MiMB 2021 (vol 2275, A. Delobel, Ed)



Check for updates

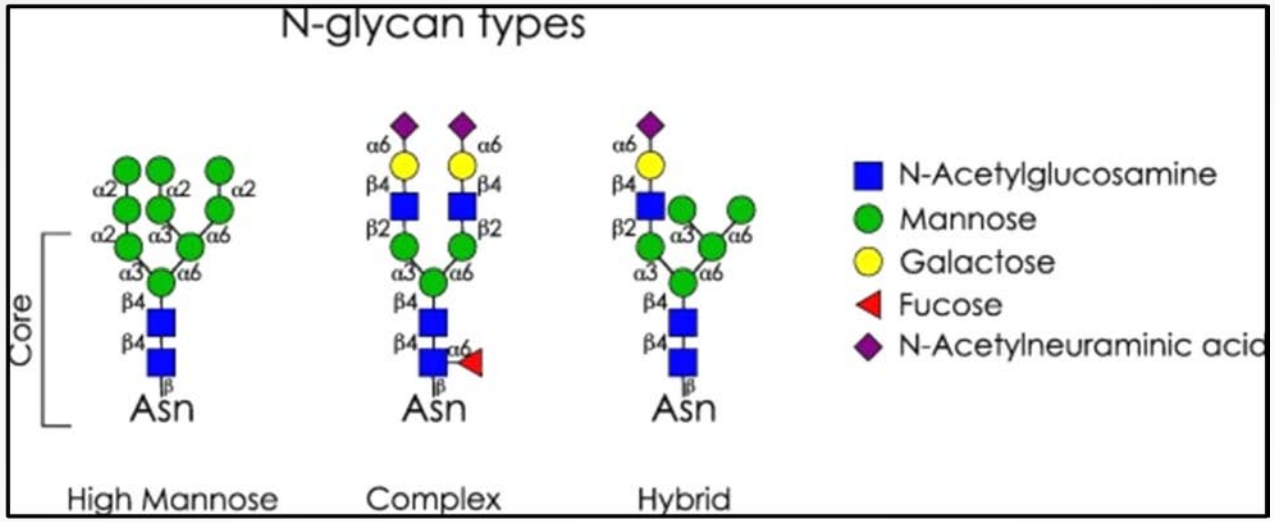
Chapter 5

Fast Afucosylation Profiling of Glycoengineered Antibody Subunits by Middle-Up Mass Spectrometry

Elsa Wagner-Rousset, Olivier Colas, Stéphane Chenu, Yannis-Nicolas François, Davy Guillarme, Sarah Cianferani, Yury O. Tsybin, Jonathan Sjögren, Arnaud Delobel, and Alain Beck

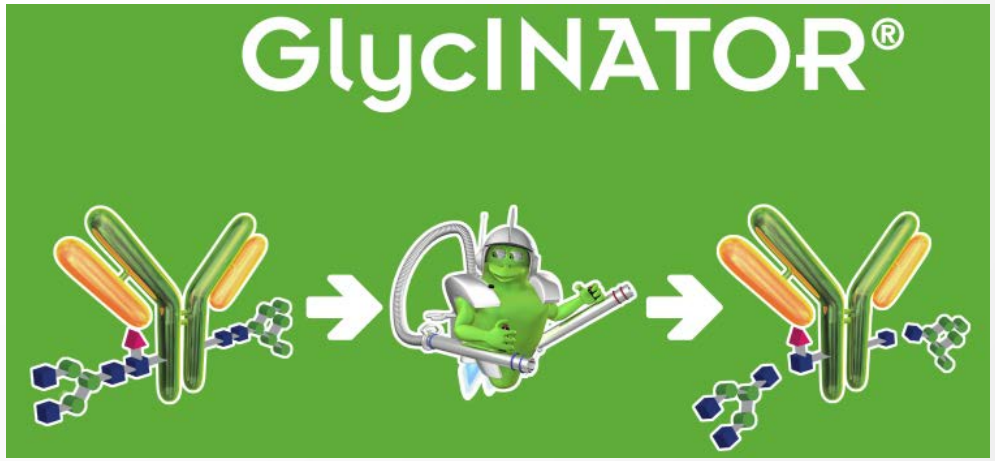
- Obinutuzumab (Glycart)
- Benralizumab (Kyowa)
- mAb A (CHO)
- mAb A (CHO + kifunensine, High mannose)

EndoS & EndoS2: study of Fc glycosylation



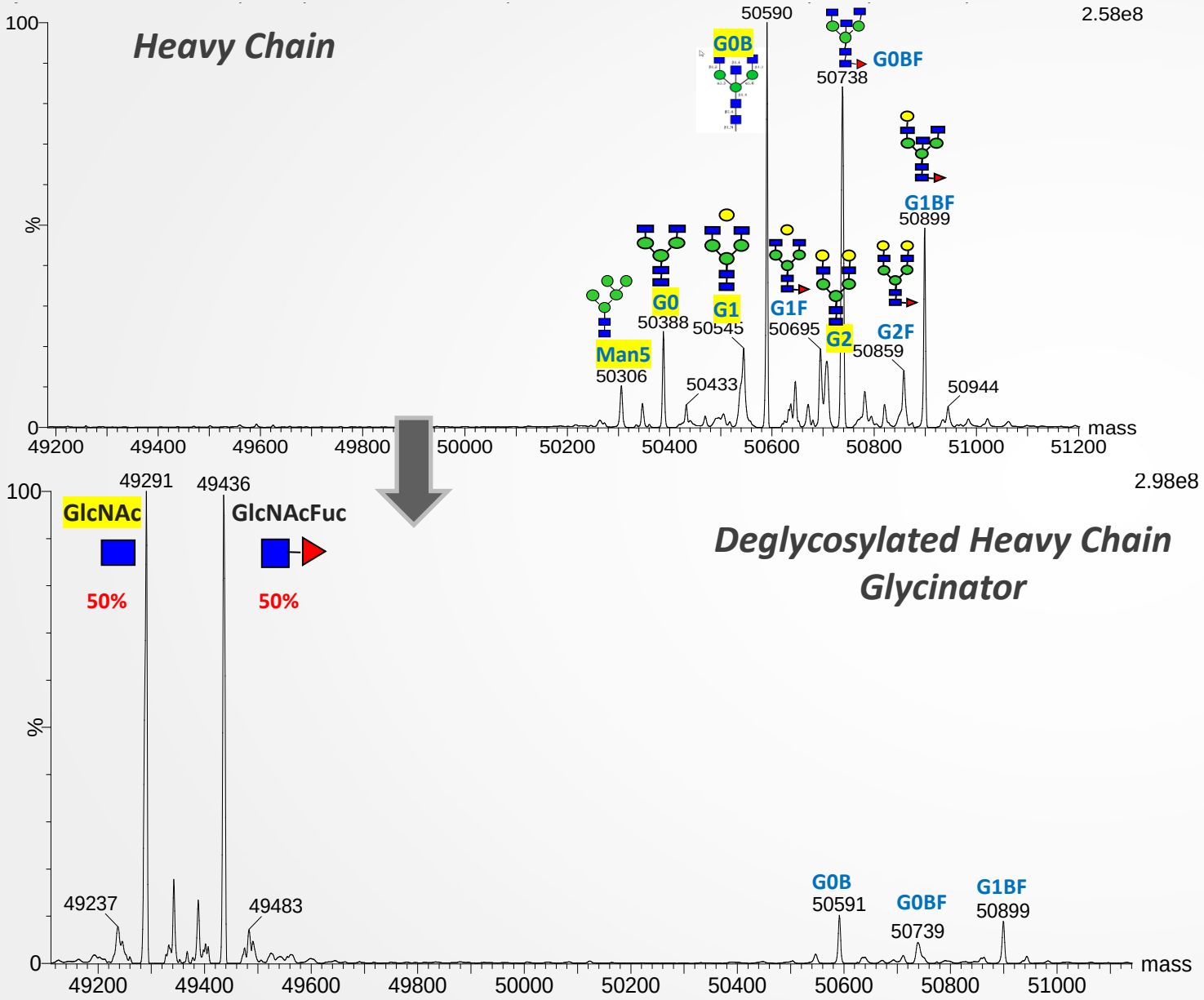
www.genovis.com

IgG-specific endoglycosidase acting on complex type N-glycans at the Fc-glycosylation site of IgG



Remove all glycoforms on IgG:
high-mannose, hybrid, complex, and bisecting type glycans

Obinutuzumab Heavy Chain glycoprofiling

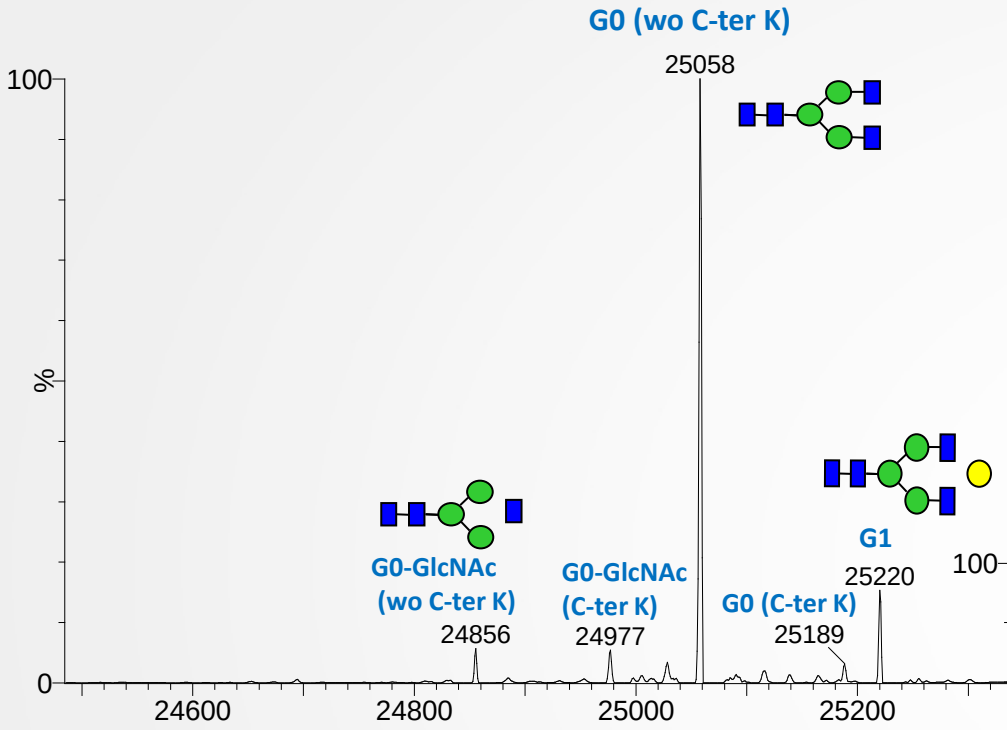


- Roche/Glycart technology
- Heavy chain includes mainly biantennary complex structures with a 3^{d} bisecting N-acetylglucosamine: G0B, G0BF, G1BF
- After deglycosylation with glycinator, the % of GlcNAc/GlcNAc-Fuc is 50/50.

- Level A-fucosylated glycans = 50%

Wagner E, Colas O, Beck A, 2021

Benralizumab subunits (IdeS/ Reduced)



MW cal. Fc/2(G0) : 25 057 Da

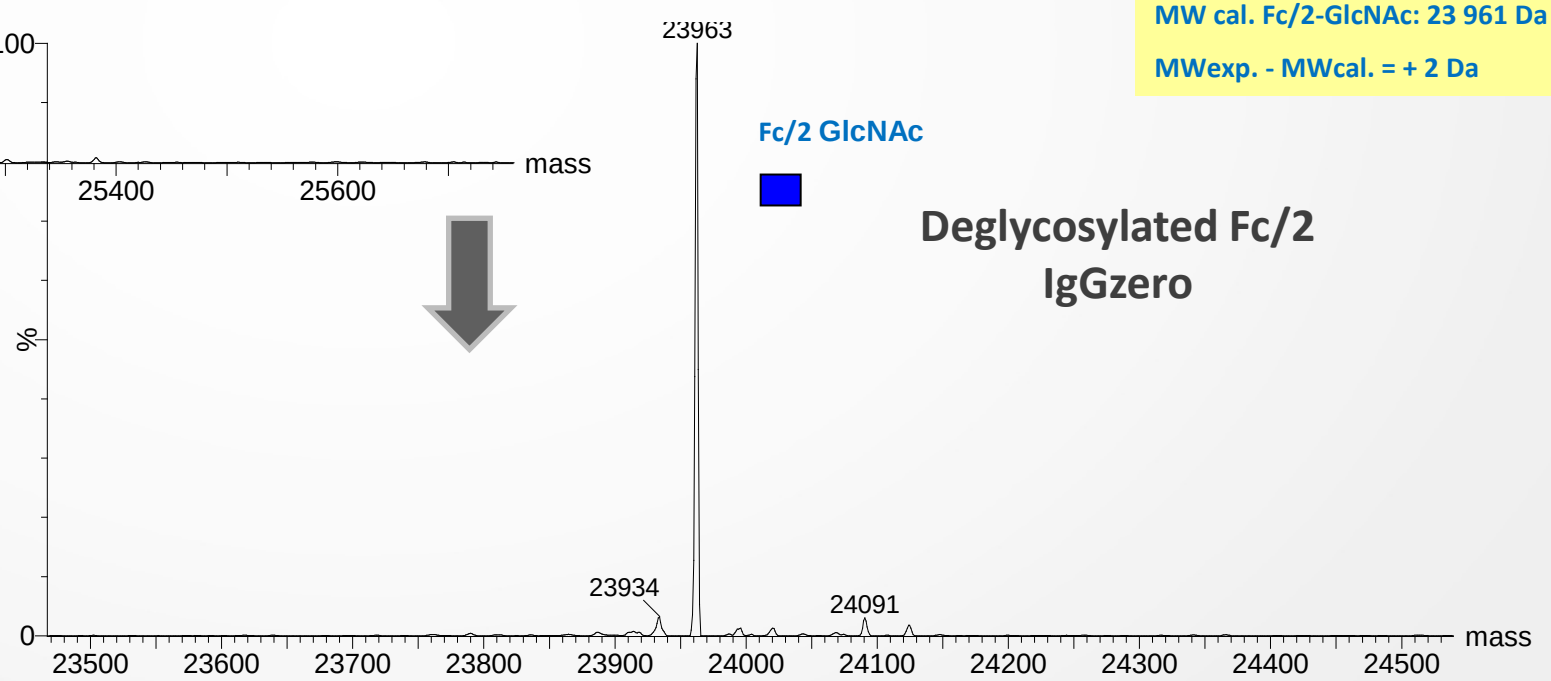
MW exp. - MW cal. = + 1Da

Level A-fucosylated glycans = 100%

- Kyowa technology
- The glycoprofile of Fc/2 displays only afucosylated biantennary complex structures G0 & G1

MW cal. Fc/2-GlcNAc: 23 961 Da

MWexp. - MWcal. = + 2 Da



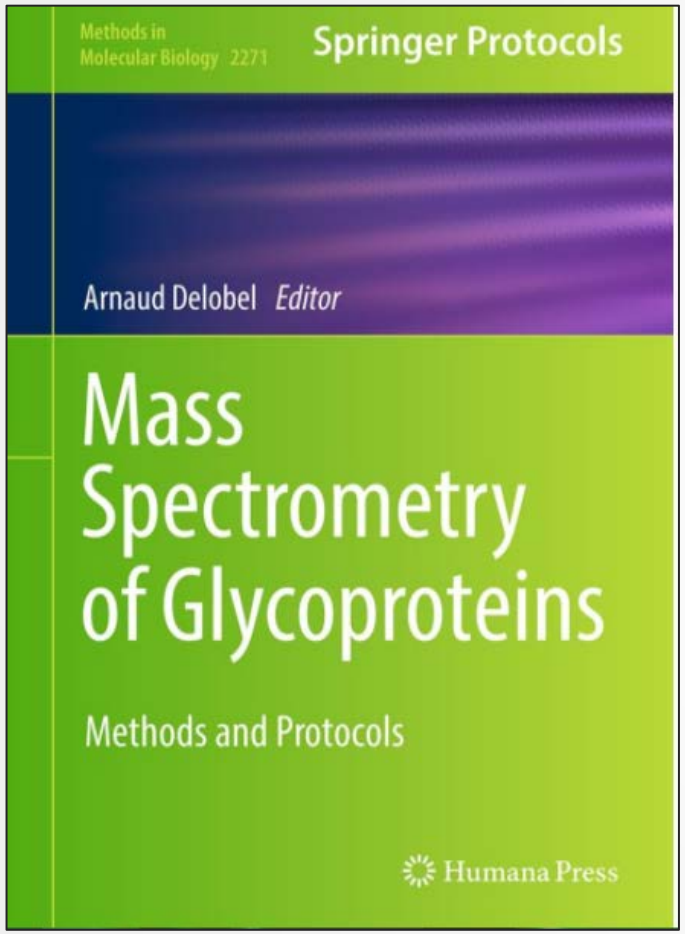
Deglycosylated Fc/2
IgGzero

2012 mogamulizumab[aFuc] (CCR4)[Jpn]
2017 benralizumab[aFuc] (IL5Ra)

2020 belantamab mafodotin[aFuc] (BCMA)

Wagner E, Colas O, Beck A 2021

MS of Glycoproteins, M&P, MiMB 2021 (vol 2275, A. Delobel, Ed)



- Y. François et al

Check for updates

Chapter 7

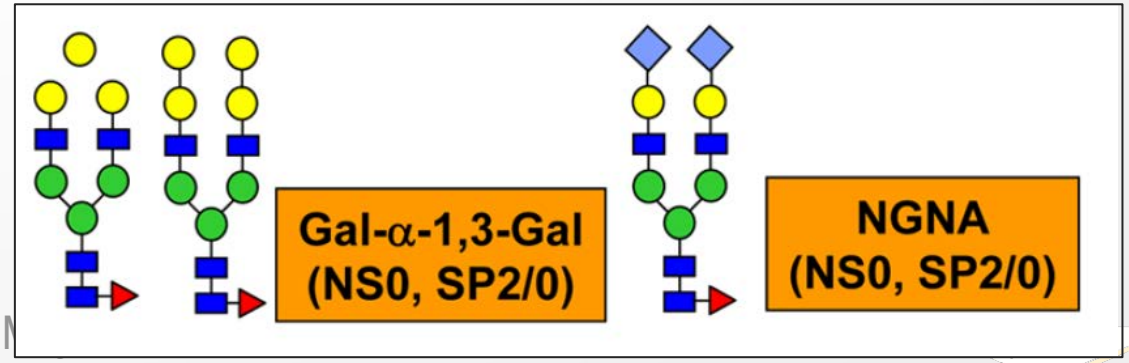
Analysis of Monoclonal Antibody Glycopeptides by Capillary Electrophoresis–Mass Spectrometry Coupling (CE-MS)

Josiane Saadé, Michael Biacchi, Jérémie Giorgetti, Antony Lechner, Alain Beck, Emmanuelle Leize-Wagner, and Yannis-Nicolas François

- Natalizumab (NS0 cells)

UNIVERSITÉ DE STRASBOURG

LSMIS



N-glycans, Middle-up HILIC-HRMS (2021)

pharmaceutics

Article

Quantitative *N*-Glycan Profiling of Therapeutic Mono… Antibodies Performed by Middle-Up Level HILIC-HR… Analysis

Bastiaan L. Duivelshof [1,2], Steffy Denorme [3], Koen Sandra [3], Xiaoxiao Liu [4], Alain Beck [5], Matthew … Davy Guillarme [1,2] and Valentina D'Atri [1,2,*]

1 Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of… CMU—Rue Michel-Servet 1, 1211 Geneva, Switzerland; Bastiaan.Duivelshof@unig… Davy.Guillarme@unige.ch (D.G.)
2 School of Pharmaceutical Sciences, University of Geneva, CMU—Rue Michel-Serv… 1211 Geneva, Switzerland
3 Research Institute for Chromatography (RIC), President Kennedypark 26, 8500 Ko… steffy.denorme@RIC-group.com (S.D.); koen.sandra@RIC-group.com (K.S.)
4 Waters Corporation, 34 Maple Street, Milford, MA 01757-3696, USA; Xiaoxiao_Liu… Matthew_lauber@waters.com (M.A.L.)
5 IRPF—Centre d'Immunologie Pierre-Fabre (CIPF), 5 Avenue Napoléon III, 60497 S… France; alain.beck@pierre-fabre.com
* Correspondence: valentina.datri@unige.ch; Tel.: +41-22-379-3358

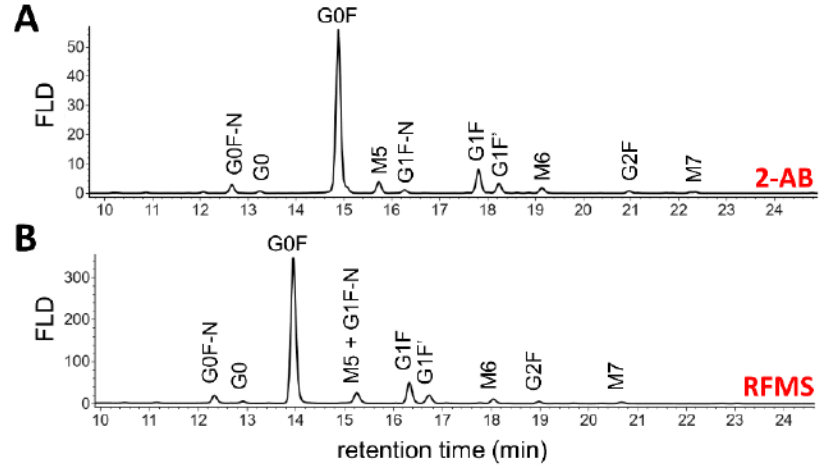


Figure 1. Fluorescence chromatograms of HILIC separated *N*-glycans of adalimumab after 2-AB (**A**) and RFMS (**B**) labelling. Different gradient profiles and mobile phase compositions were used for the HILIC analysis of the 2-AB and RFMS labelled glycans (see Section 2.4). Glycan identification based on calculated GU values and/or MS detection.

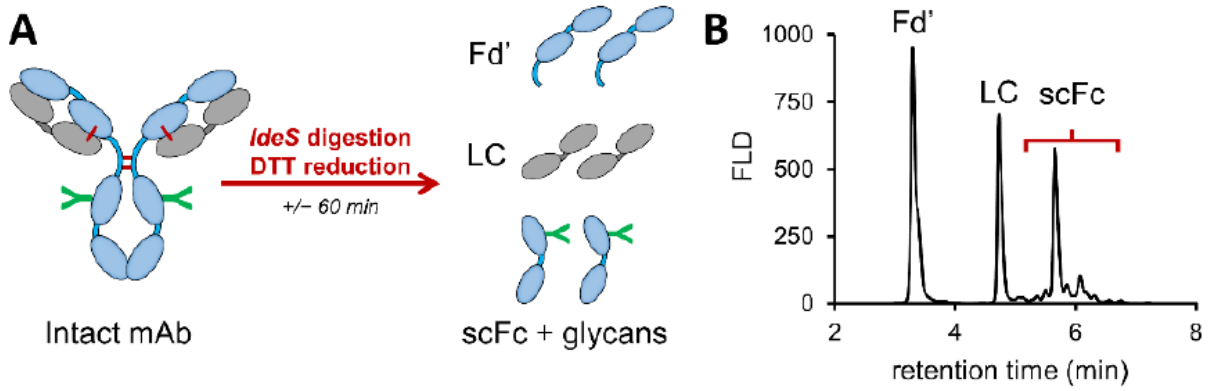


Figure 2. Middle-up HILIC-MS analysis of *IdeS* digested and DTT reduced adalimumab. (**A**) Sample preparation procedure for the digestion and reduction in intact mAb to protein subunits. (**B**) FLD chromatogram displays the separation of the Fd', LC and scFc subunits. See Table S1 for detailed retention times and mass assignment.

NISTmAb – Glyco-NIST collab. Study (MCP 2020)

NIST Interlaboratory Study on Glycosyl
Comparison of Results fron

NIST Interlaboratory Study

76 Participants, 103 Datasets

Highlights

- A broad-based interlaboratory study of the glycosylation of a reference antibody: NISTmAb.
- 103 reports were received from 76 diverse laboratories worldwide.
- Analysis involved two samples, the NISTmAb and an enzymatically modified sample, enabling within-lab separation of random and systematic errors using the "Youden two-sample" method.
- Consensus values were derived and similar performance across all experimental methods was noted.

NIST Interlaboratory Study on Glycosylation Analysis of Monoclonal Antibodies: Comparison of Results from Diverse Analytical Methods.

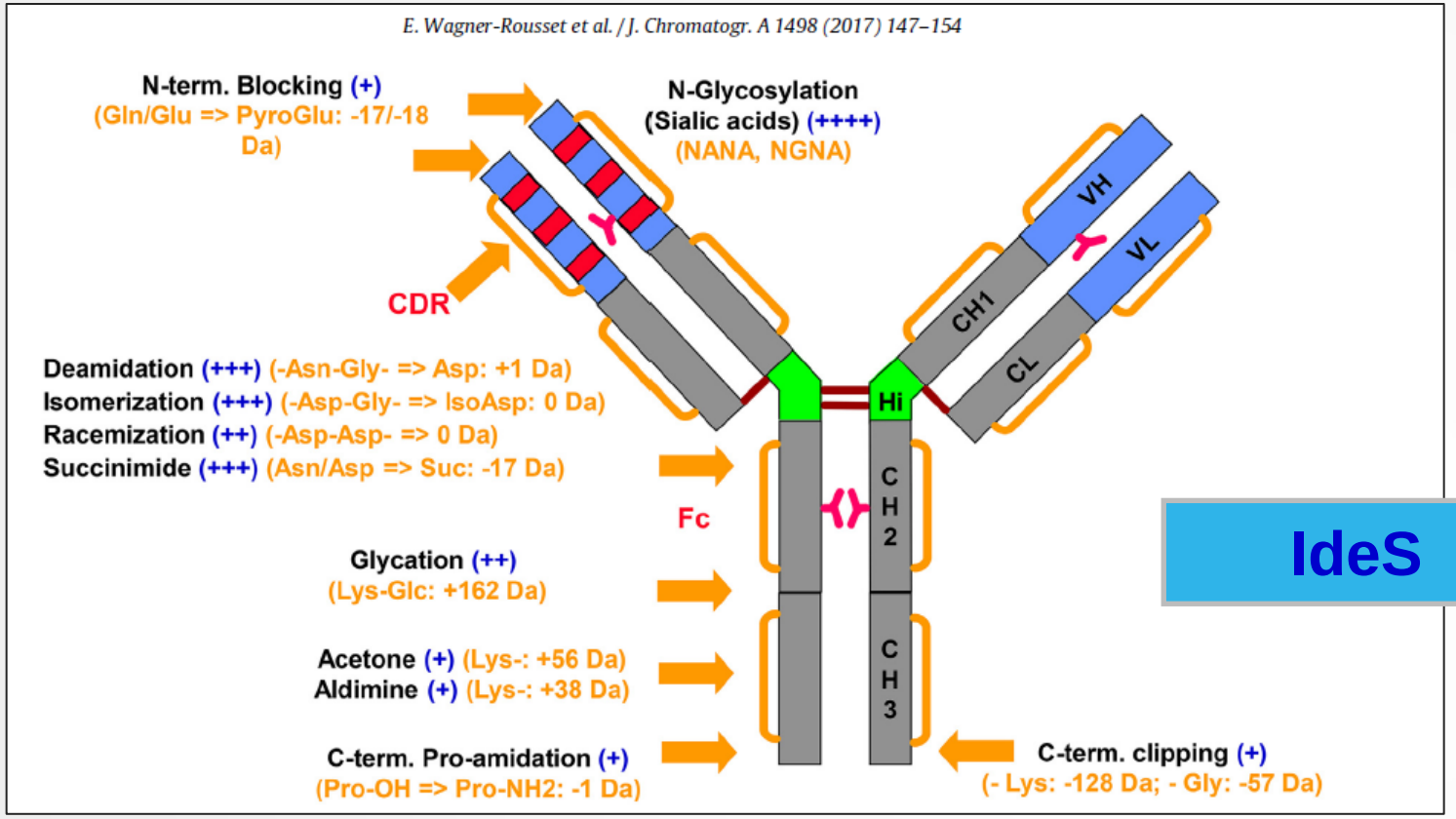
De Leoz MLA, Duewer DL, Fung A, Liu L, Yau HK, Potter O, Staples GO, Furuki K, Frenkel R, Hu Y, Sosic Z, Zhang P, Altmann F, Gruber C, Shao C, Zaia J, Evers W, Pangelley S, Suckau D, Wiechmann A, Resemann A, Jabs W, **Beck A**, Froehlich JW, Huang C, Li Y, Liu Y, Sun S, Wang Y, Seo Y, An HJ, Reichardt NC, Ruiz JE, Archer-Hartmann S, Azadi P, Bell L, Lakos Z, An Y, Cipollo JF, Pučić-Baković M, Štambuk J, Lauc G, Li X, Wang PG, Bock A, Hennig R, Rapp E, Creskey M, Cyr T, Nakano M, Sugiyama T, Leung PA, Link-Lenczowski P, Jaworek J, Yang SJ,

Leize-Wagner E, Maier S, Zeck A, Heck AJR, Yang Y, Haselberg R, Yu YQ, Alley W, Leone JW, Yuan H, Stein SE.

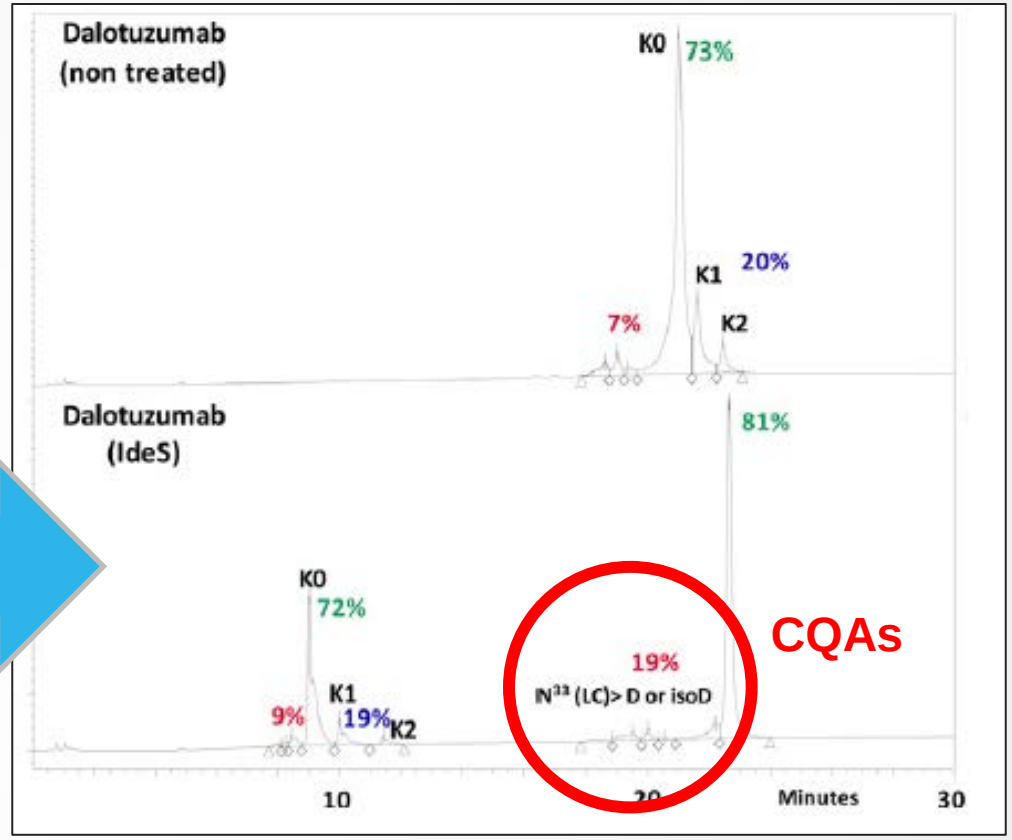
Mol Cell Proteomics. 2019 Oct 7. pii: mcp.RA119.001677. doi: 10.1074/mcp.RA119.001677. [Epub ahead of print]
PMID: 31591262 **Free Article**

- **Leoz L, Duewer D, Beck A & 100+ scientists. Mol Cell Proteomics 2020**

(2.5) IgGs charge variants (CEX, IEF, icIEF, 2D-LC-MS)



IdeS



- Beck A, Wagner E, Cianferani S et al. Anal Chem 2013
- Fekete S, Beck A, Veuthey JL, Guillarme D. JPBA 2015 (CEX)
- Fekete S, Beck A, Guillarme D. JPBA 2015 (IEX)
- Stoll D, Beck A et al. Anal Chem 2015 (2D-LC-MS)
- Stoll D, Beck A et al. mAbs 2016 (2D-LC-MS)

- Wagner-Rousset E, Guillarme D

Beck A et al. J Chrom A 2017

Charge variants & pI: icIEF (Protein simple) & CEX (2017)

Journal of Chromatography B 1065–1066 (2017) 119–128

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

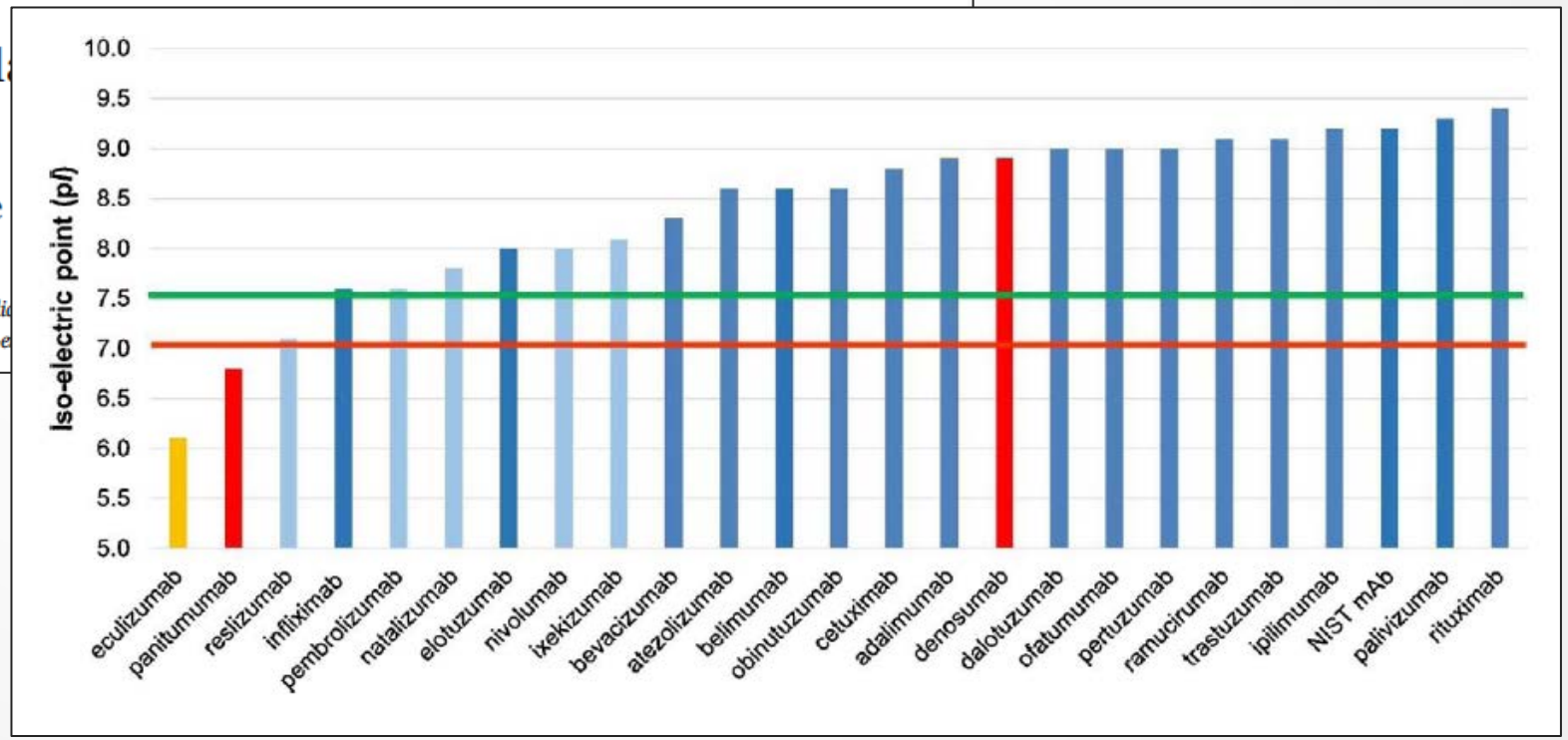
UNIVERSITÉ DE GENÈVE

- icIEF
- CEX

Determination of isoelectric points and rel
therapeutic monoclonal antibodies

Alexandre Goyon[a,1], Melissa Excoffier[b,1], Marie-Claire
Szabolcs Fekete[a], Davy Guillarme[a,*], Alain Beck[b]

[a] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Centre Médi
[b] Center of Immunology Pierre Fabre, 5 Avenue Napoléon III, BP 60497, 74160 Saint-Julien-e



- 23 FDA/EMA approved IgGs
- Extended range of pIs (6.2-9.5)
- IgG1, IgG2, IgG2/4, IgG4
- Calculated vs exp. pIs (icIEF)

Ultra-short IEX columns for charge variants (JCA 2021)

Journal of Chromatography A 1657 (2021) 462568

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Ultra-short ion-exchange columns for fast charge variants analysis of therapeutic proteins

Jose Antonio Navarro-Huerta[a], Amarande Murisier[b,c], Jennifer M. Nguyen[d], Matthew A. Lauber[d], Alain Beck[e], Davy Guillarme[b,c], Szabolcs Fekete[b,c,*]

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ARTICLE INFO

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Available online 22 September 2021

ABSTRACT

The purpose of this work was to
for ion exchange chromatography
10, 15, 20 and 50 mm and packe
pH and salt gradient modes of el
reversed phase liquid chromatography (RPLC) mode, an "on-off" retention mechanism was observed in
IEX for therapeutic proteins and their fragments (25–150 kDa range)

Highlights

- prototype ultra-short ion exchange columns were developed
- the ideal column length was experimentally determined
- elution mechanism of mAbs were studied in pH and salt gradient modes
- separations of intact and IdeS digested mAbs were performed in 1-2 min

CEX-native MS for charge variants (JCA 2021)

Journal of Chromatography A 1655 (2021) 462499

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Towards a simple on-line coupling of ion exchange chromatography and native mass spectrometry for the detailed characterization of monoclonal antibodies

Amarande Murisier[a,b], Bastiaan L. Duivelshof[a,b], Szabolcs Fekete[a,b], Julien Bourquin[c], Andrew Schmudlach[c], Matthew A. Lauber[c], Jennifer M. Nguyen[c], Alain Beck[d], Davy Guillarme[a,b], Valentina D'Atri[a,b,*]

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[b] School of Pharmaceutical Sciences, University of Geneva, CMU-Rue Michel Servet 1, 1211 Geneva 4, Switzerland
[c] Waters Corporation, 34 Maple Street, Milford, Massachusetts 01757-3696, United States
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CEX optimization: column + eluant (JCA 2020)

Journal of Chromatography A 1626 (2020) 461350

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Impact of the column on
chromatography, a practi

Evelin Farsang[a], Krisztián Horvá
Davy Guillarme[e], Szabolcs Feket

[a] Department of Analytical Chemistry, University of Pan
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[c] Waters Corporation, 34 Maple Street, Milford, MA 017
[d] Current Address: Bristol Myers Squibb, 38 Jackson Rd,
[e] Institute of Pharmaceutical Sciences of Western Switze

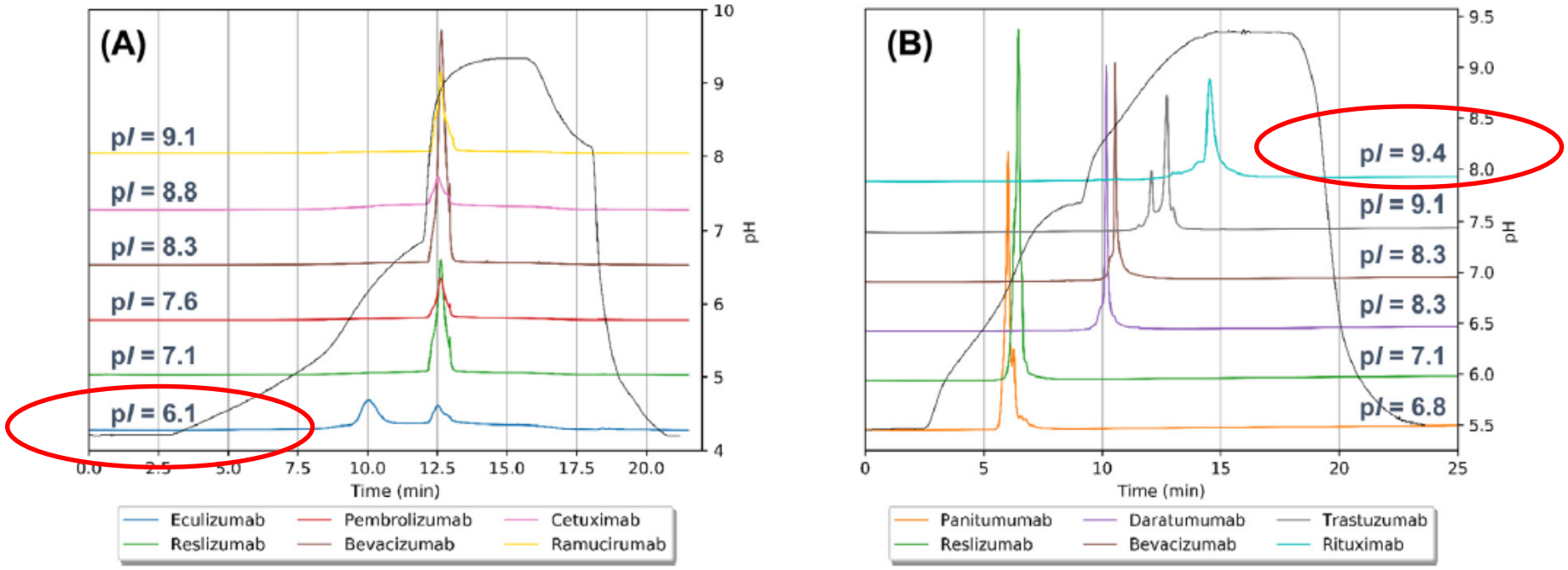

Fig. 5. Chromatograms of intact mAbs eluted from a Thermo ProPac column withCA/CHES/NaOH pH gradient buffer (A) and with MES/DAP pH gradient buffer (B).

- **S. Fekete, D. Guillarme et al**

Non-denaturing (2D-)LC-MS: IEX, SEC, HIC-MS (JPBA 2020)

Journal of Pharmaceutical and Biomedical Analysis 185 (2020) 113207

Contents lists available at ScienceDirect

Journal of Pharmaceutical a

journal homepage: www.els

Review

Coupling non-denaturing chromatography
the characterization of monoclonal antibod

Evelin Farsang[a], Davy Guillarme[b], Jean-Luc Veuthey[b], A
Andrew Schmudlach[d], Szabolcs Fekete[b,*]

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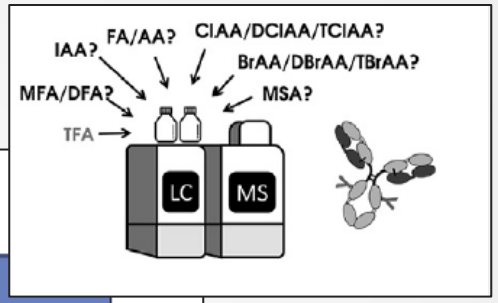
[b] Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, CMU-Rue M
[c] Center of Immunology Pierre Fabre, 5 Avenue Napoléon III, BP 60497, 74160, Saint-Julien-en-G
[d] Waters Corporation, 34 Maple Street, Milford, MA, 01757-3696, United States

- **S. Fekete, D. Guillarme et al**

Alernative mobile phase additives in LC-MS (Anal Chem Acta A 2021)



Analytica Chimica Acta 1156 (2021) 338347

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Alternative mobile phase additives for the characterization of biopharmaceuticals in liquid chromatography – Mass spectr

Honorine Lardeux [a, b], Bastiaan L. Duivelshof [a, b], Olivier Colas [c], Alain Beck [c], David V. McCalley [d], Davy Guillarme [a, b], Valentina D'Atri [a, b, *]

[a] Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, CMU-Rue Michel Servet 1, 1211, Geneva 4,
[b] School of Pharmaceutical Sciences, University of Geneva, CMU-Rue Michel Servet 1, 1211, Geneva 4, Switzerland
[c] IRPF - Centre D'Immunologie Pierre-Fabre (CIPF), 5 Avenue Napoléon III, BP 60497, Saint-Julien-en-Genevois, France
[d] Centre for Research in Biosciences, University of the West of England, Frenchay, Bristol, BS16 1QY, UK

HIGHLIGHTS

- Fifteen mobile phase additives are tested for mAb analysis in RPLC- and HILIC-MS.
- A first evaluation is performed at chromatographic level by using a FLD detector.
- As alternative to TFA, four additives are selected in RPLC mode and one in HILIC mode.
- Performance of selected additives are investigated in MS after volatility assessment.

Capillary Electrophoreris + MS (CE-MS) (JPBA 2020)

Journal of Pharmaceutical and Biomedical Analysis 182 (2020) 113107

Contents lists available at ScienceDirect

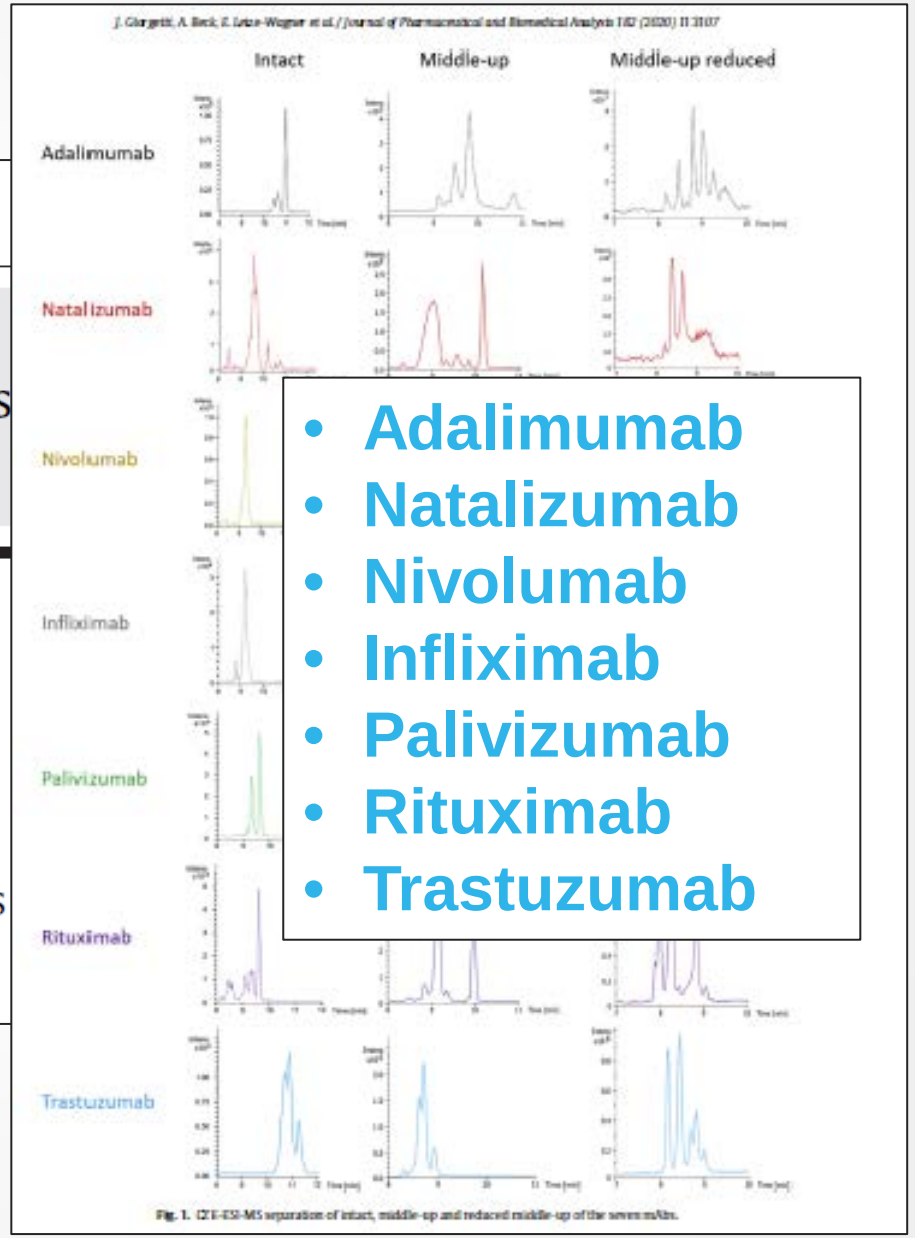
Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Combination of intact, middle-up and bottom-up levels to characterize 7 therapeutic monoclonal antibodies by capillary electrophoresis – Mass spectrometry

Jérémie Giorgetti[a], Alain Beck[b], Emmanuelle Leize-Wagner[a], Yannis-Nicolas François

[a] *Laboratoire de Spectrométrie de Masse des Interactions et des Systèmes (LSMIS) UMR 7140 (Unistra-CNRS), Université de Strasbourg, France*
[b] *Centre d'Immunologie Pierre Fabre, Saint-Julien-en-Genevois, France*



- Adalimumab
- Natalizumab
- Nivolumab
- Infliximab
- Palivizumab
- Rituximab
- Trastuzumab

- Y. François et al

antibodies

MDPI

Antibodies (2019)

Dr. Hongcheng Liu

Review

Macro- and Micro-Heterogeneity of Natural and Recombinant IgG Antibodies

Alain Beck [1,*] and **Hongcheng Liu** [2,*]

[1] Biologics CM
74160 Saint-J
[2] Anokion, 50
* Correspond

Received: 22 Dec

Abstract: Reco
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of the drug and
of the current u
recombinant m

Keywords: cri
product profile

Pierre Fa

Table 1. Micro-heterogeneity natural IgGs and recombinant mAbs.

Modifications	Natural	Recombinant	Resulting Heterogeneity
N-terminal modifications			
PyroGlu	100% pyroGlu	Varied levels	Mass, charge for Gln to pyroGlu
Truncation	Not expected	Rare and low	Mass
Signal peptides	Not expected	Low	Mass and charge
Asn deamidation	Substantial level	Common, varied levels	Mass and charge
Asp isomerization	Not expected	Common, varied levels	Charge and hydrophobicity
Succinimide	Not expected	Common, varied levels	Mass, charge, and hydrophobicity
Oxidation	Low	Met, Trp, Cys, His	Mass and hydrophobicity
Cysteine related modifications			
Free cysteine	Low	Low	Mass, charge and hydrophobicity
Alternative disulfide bond linkage	Common	Common	Charge
Trisulfide bond	Extremely low	Low	Mass and charge
Thioether	Low	Low	Mass
Glycosylation	Common	Common	Mass and charge
Glycation	Common	Common	Mass and charge
C-terminal modifications			
C-terminal Lys	Complete removal	Common, varied levels	Mass, charge and hydrophobicity
C-terminal modifications	Not detected	Low varied levels	Mass and charge

(I) Dalotuzumab (h7C10/MK0646) – 2005-2016

THOMSON REUTERS

Current Opinion in Molecular Therapeutics 2010 12(3):363-371
© Thomson Reuters (Scientific) Ltd ISSN 2040-3445

DRUG PROFILE

Dalotuzumab, a recombinant humanized mAb targeted against IGFR1 for the treatment of cancer

Mario Scartozzi[1]*, Maristella Bianconi[2], Elena Maccaroni[2], Riccardo Giampieri[2], Rossana Berardi[1] & Stefano Cascinu[1]

Addresses
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Email: mariascartozzi@gmail.com

[2]Università Politecnica delle Marche, Scuola di Specializzazione in Oncologia, Via conca, 60020, Ancona, Italy

*Towhom correspondence should be addressed

Dalotuzumab (MK-0646; h7C10), being developed by Merck & Co Inc under license from Pierre Fabre SA, is a recombinant humanized IgG1 mAb against the IGFR1 for the potential intravenous treatment of cancer. Preclinical studies have demonstrated that dalotuzumab acts by inhibiting IGF-1- and IGF-2-mediated tumor cell proliferation, IGFR1 autophosphorylation and Akt phosphorylation. In multiple cancer cell lines and in mouse xenograft models, dalotuzumab displayed significant antitumor activity. In particular against NSCLC and breast cancer. In addition, coadministration of dalotuzumab with other anticancer agents, such as taxanes, enhanced the in vitro and in vivo antitumor activity of dalotuzumab. Preliminary data from phase I clinical trials suggest that dalotuzumab is safe, well tolerated and significantly inhibits tumor proliferation. At the time of publication, several clinical trials evaluating dalotuzumab, alone and in combination with other anticancer agents, were ongoing in patients with various types of solid tumor and in patients with multiple myeloma. Although preliminary results appear promising, only future clinical and translational data will clarify the best clinical setting and treatment combinations for the optimal use of dalotuzumab in clinical practice.

JNCI J Natl Cancer Inst (2015) 107(12): djv258

doi:10.1093/jnci/djv258
First published online September 23, 2015
Article

OXFORD

ARTICLE

A Randomized Phase II/III Study of Dalotuzumab in Combination With Cetuximab and Irinotecan in Chemorefractory, KRAS Wild-Type, Metastatic Colorectal Cancer

Francesco Sclafani, Tae Y. Kim, David Cunningham, Tae W. Kim, Josep Tabernero, Hans J. Schmoll, Jae K. Roh, Sun Y. Kim, Young S. Park, Tormod K. Guren, Eliza Hawkes, Steven J. Clarke, David Ferry, Jan-Erik Frödin, Mark Ayers, Michael Nebozhyn, Clare Peckitt, Andrey Loboda, David J. Mauro, David J. Watkins

Pierre Fabre

MERCK

(II) Telisotuzumab (Hz224G4, ABT-700; cMet) - 2016

IJC
International Journal of Cancer
Int. J. Cancer: 139, 1851–1863 (2016)

A novel antagonist anti-cMet antibody with antitumor activities targeting both ligand-dependent and ligand-independent c-Met receptors

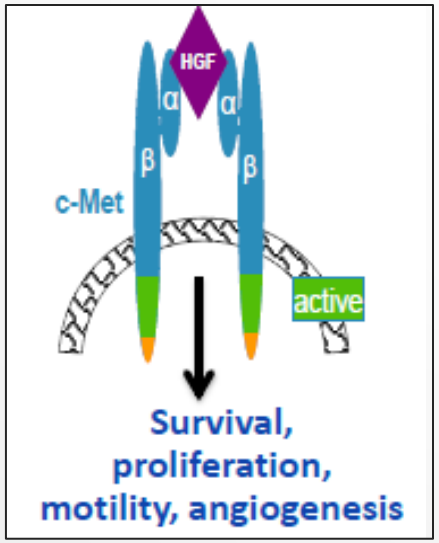
Alexandra Gonzalez, Matthieu Broussas, Charlotte Beau-Larvor, Jean-François Haeuw, Nicolas Boute, Alain Robert, Thierry Champion, Alain Beck, Christian Bailly, Nathalie Corvaïa and Liliane Goetsch

Centre D'Immunologie Pierre Fabre 5, IRPF, Av Napoléon III, F-74164, Saint-Julien-en-Genevois, France

Pierre Fabre

abbvie

NCT01472016



BMC Cancer
Wang et al. BMC Cancer (2016) 16:105
DOI 10.1186/s12885-016-2138-z

RESEARCH ARTICLE Open Access

Anti-c-Met monoclonal antibody ABT-700 breaks oncogene addiction in tumors with *MET* amplification

Jieyi Wang[1,4*], Liliane Goetsch[2], Lora Tucker[1], Qian Zhang[1], Alexandra Gonzalez[2], Kedar S. Vaidya[1], Anatol Oleksijew[1], Erwin Boghaert[1], Minghao Song[3], Irina Sokolova[3], Ekaterina Pestova[3], Mark Anderson[1], William N. Pappano[1], Peter Ansell[1], Anahita Bhathena[1], Louie Naumovski[4], Nathalie Corvaia[2] and Edward B. Reilly[1]

(III) Hz515H7 mAb (CXCR4): Ph I RRMM (2018)

www.oncotarget.com Oncotarget, 2018, Vol. 9, (No. 35), pp: 23890-23899

Research Paper

Phase I dose-escalation study of F50067, a humanized anti-CXCR4 monoclonal antibody alone and in combination with lenalidomide and low-dose dexamethasone, in relapsed or refractory multiple myeloma

Guillemette Fouquet[1,*], Stéphanie Guidez[2,11,*], Valentine Richez[2], Anne-Marie Stoppa[3], Christophe Le Tourneau[4], Margaret Macro[5], Cécile Gruchet[2], Arthur Bobin[2], Niels Moya[2], Thomas Syshenko[2], Florence Sabirou[2], Anthony Levy[2], Paul Franques[2], Hélène Gardeney[2], Lionel Karlin[6], Lotfi Benboubker[7], Monia Ouali[10], Jean-Claude Vedovato[10], Pierre Ferre[10], Mariya Pavlyuk[10], Michel Attal[8], Thierry Facon[9] and Xavier Leleu[2,11]

Pierre Fabre

- **Fouquet G et al, Oncotarget 2018**

(IV) Vista IO mAb: W0180 (Nov 2020)

Pierre Fabre initiates a "First in Human" clinical trial for an innovative monoclonal antibody (W0180) targeting the VISTA checkpoint in patients with solid tumors

Pierre Fabre

News provided by Pierre Fabre Nov 09, 2020, 09:00 ET

TOULOUSE, France, Nov. 9, 2020 /PRNewswire/ -- Pierre Fabre announced today the initiation of an international Phase I clinical study in patients with relapsed or refractory solid tumors for its investigational product W0180, an innovative monoclonal antibody targeting VISTA, developed by Pierre Fabre Medical Care R&D teams. The study started at the University Clinic of Navarra, Spain, under the supervision of Pr. Ignacio Melero, Immunologist and Senior Investigator at Centro de Investigación Médica Aplicada (CIMA).

This clinical research is led by Principal Investigator Pr. Aurelien Marabelle of the Gustave Roussy Cancer Institute (Villejuif, France). Pr. Marabelle is the Clinical Director of the Cancer Immunotherapy Program at Gustave Roussy and Senior Medical Oncologist within its Drug Development Department (DITEP). The study will involve other sites in France and Spain, including at the Toulouse University Hospital (IUCT) located at the Toulouse-Oncopole Campus.

(V) IGF1R mAb, TED, ValenzaBio (Apr/ June 21)

License and Commercialization agreement between Pierre Fabre and ValenzaBio on an anti-IGF-1R antibody for the development of a novel treatment in Thyroid Eye Disease (TED)

Pierre Fabre

VALENZABio

08 Apr, 2021, 12:00 BST

BESTHESDA, Maryland and CASTRES, France, April 8, 2021 /PRNewswire/ -- The US biopharmaceutical company ValenzaBio and the French pharmaceutical group Pierre Fabre announced today the signing of a license agreement of a preclinical insulin-like growth factor-1 receptor (IGF-1R) antagonist antibody, for the treatment of Thyroid Eye Disease (TED), an endocrine disease with unmet medical need. ValenzaBio received from Pierre Fabre the worldwide and exclusive rights to develop and commercialize, outside of oncology, the anti-IGF-1R antibody, discovered by Pierre Fabre at its Center of immunology located in Saint-Julien-en-Genevois (France), with the aim of treating patients suffering from TED. Other indications in rare diseases could also be developed by ValenzaBio. In parallel, Pierre Fabre is pursuing the development of its anti-IGF-1R ADC program (W0101) in oncology.

biopharma-reporter.com/Article/2021/06/23/Lonza-to-provide-manufacturing-for-ValenzaBio-at-China-site

Lonza to provide manufacturing for ValenzaBio from China facility

By Ben Hargreaves

23-Jun-2021 - Last updated on 23-Jun-2021 at 10:05 GMT

Lonza Biologics Guangzhou facility © Lonza

RELATED TAGS: fill/finish, Lonza, Drug substances, China, bioreactor

Lonza will provide drug substance and product manufacturing for the biotech's anti-IGF-1R antibody.

lonigutamab

IGF1R mAb, TED, ValenzaBio (31.03.22)

ValenzaBio Announces FDA Clearance of Investigational New Drug Application for VB421, an Anti-IGF-1R Monoclonal Antibody for the Treatment of Thyroid Eye Disease

Category: **Antibodies**

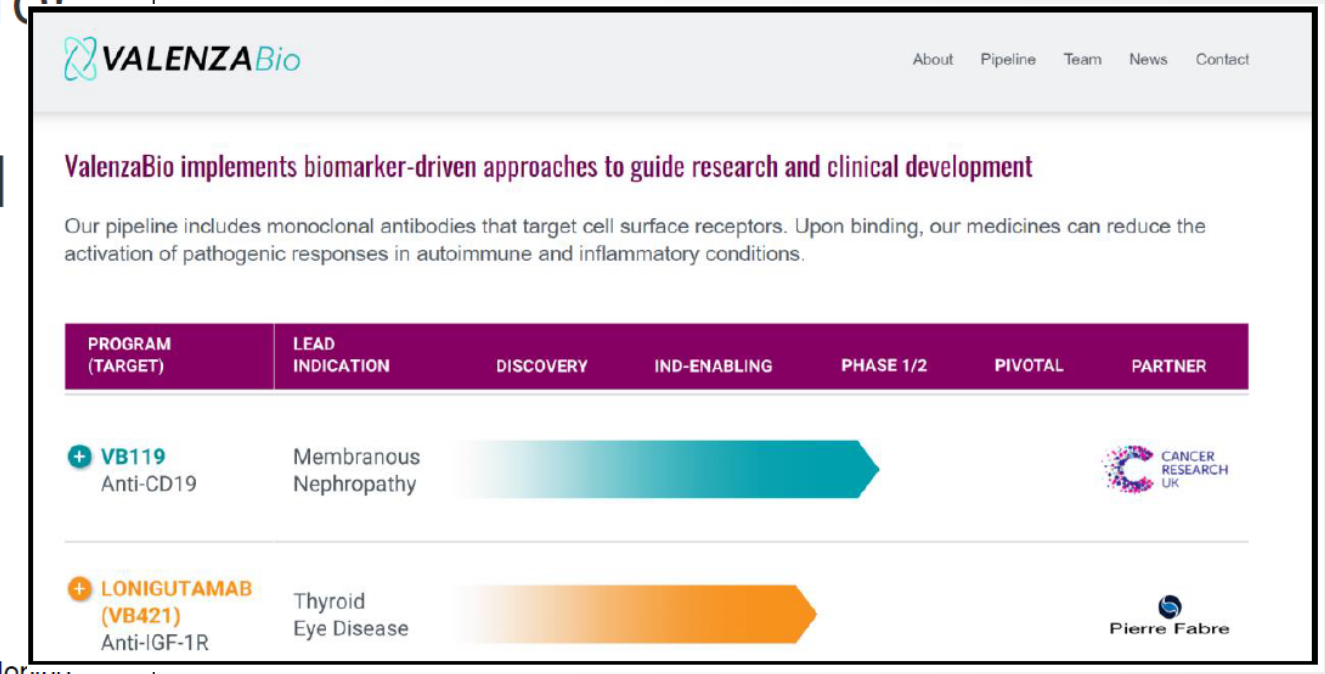
Published on Friday, 01 April 2022 09:56

Company plans to initiate single ascending dose, first-in-human study imminently

Early single dose cohorts to include subcutaneous formulation

Topline results from the Phase 1a expected in Q3 2022

BETHESDA, MD, USA I March 31, 2022 I ValenzaBio, Inc., a biopharmaceutical company developing monoclonal antibody (mAb) therapeutics for autoimmune and inflammatory indications, today announced that its investigational new drug (IND) application for its lead drug candidate, VB421, for the treatment of thyroid eye disease (TED), has been cleared by the U.S. Food and Drug Administration (FDA) for clinical evaluation. VB421 is a potential best-in-class mAb targeting IGF-1R, which plays a central role in the pathogenesis of TED.



R&D PHARMA

Pierre Fabre

(3) State of the art analytical methods for BsAbs, Fc-fusion proteins & peptides, ADCs

(3.1)

EDITORIAL

mAbs 3:3, 1-2; May/June 2011; © 2011 Landes Bioscience

The amazing, multipurpose antibody

Alain Beck[1] and Janice M. Reichert[2,*]

[1]Centre d'Immunologie Pierre Fabre; Saint Julien en Genevois, France; [2]Landes Bioscience; Austin, TX USA

Bi and tri-specific Abs (5/119)

2009 catumaxomab ***(EpCAM x CD3), withdrawn 2017***

2014 blinatumomab[scFv-scFv] **(CD19 x CD3)**

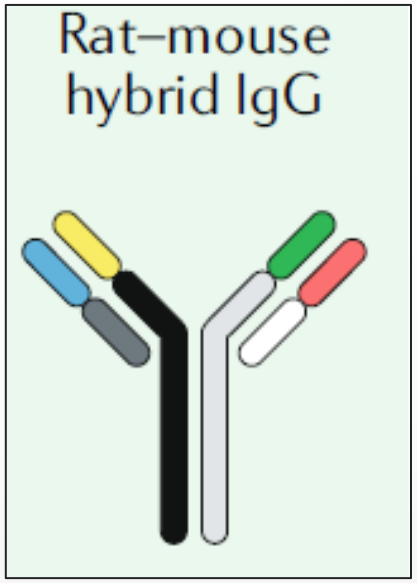
2017 emicizumab[G4bs] **(FIXa x FX)**

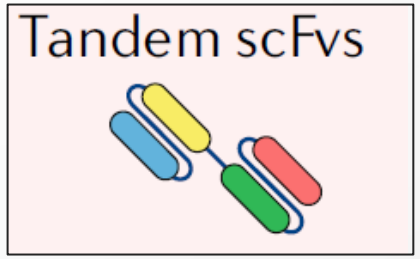
2021 amivantamab[G1bs] **(cMet x EGFR)**

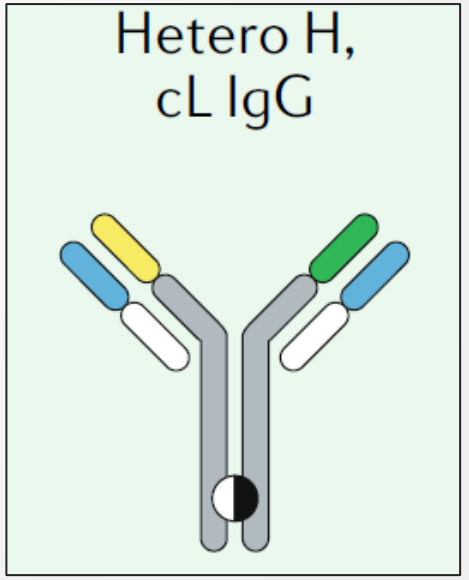
2022 faricimab[G1bs;kl] **(Ang-2 x VEGF-A)**

2022 Tebentafusp[TCR-scFv] **(GP100 x CD3)**

- **Labrijn A et al, Nature Rev DD 2019**
- **Duivelshof B, Beck A et al, Talenta 2022**

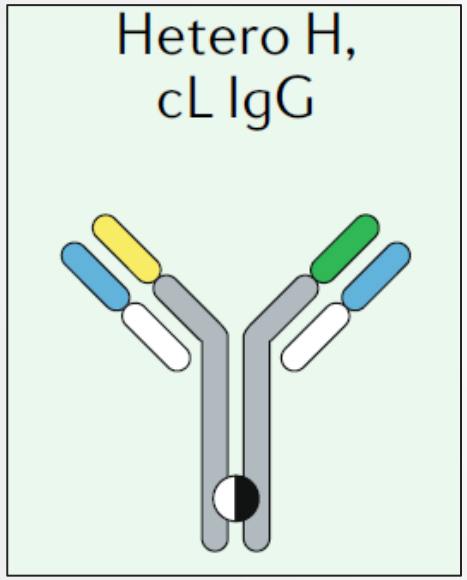


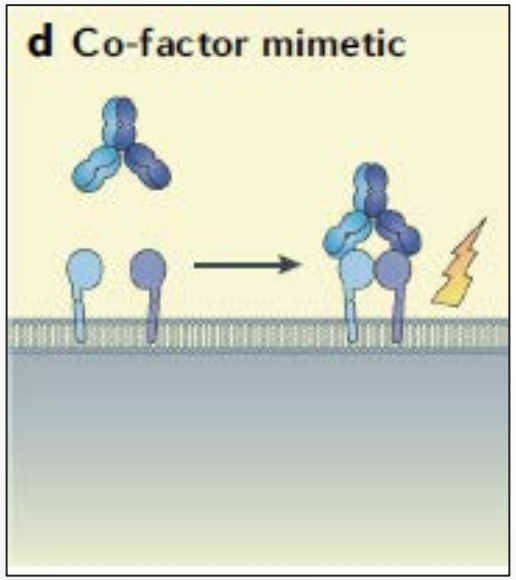




Emicizumab-kxwh (Helimbra®, Chugai/Roche 2017)

Emicizumab, Hemlibra, ACE910, RO5534262 (Chugai, Roche)	FIXa x FX and/ or FXa	• Hetero H, cL IgG4 (#11, ART-Ig, ASYM, 1 + 1) • Hetero HH: E356K x K439E, HL-pairing: cL, S228P (hinge-stabilization), G446del-K447del (reduction charge-heterogeneity), K196Q-F296Y (pI-engineering), H435R (purification)	Routine prophylaxis of patients with haemophilia A with and without FVIII inhibitors





- 40,000 asym. bsAbs from 200 IgGs (anti-FIXa or FX)
- bsAbs hits with FVIII mimetic prop: ~0.3%
- Selection of 1 LC (chain-association issue)
- LC further optimized by FR and CDR shuffling
- Additional rounds of optimization included:
  - humanization to reduce immunogenicity risk
  - CDR mutagenesis to further increase enzyme activity
  - Charge engin. of variable region (impr. solubility & PK)
  - pI engineering (bsAb homodimeric purification, CEX)
  - HC C-terminal aa deletion to reduce basic charge variants
  - De-amidation hot spot removal (CDR)

- Sampei Z et al, PLoS One 2013
- Labrijn A et al, Nature Rev DD 2019

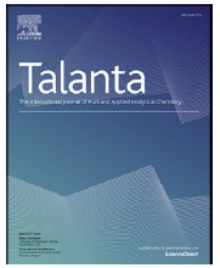
LC-MS for BsAbs: emicizumab (Talenta 2022)

Talanta 236 (2022) 122836

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta



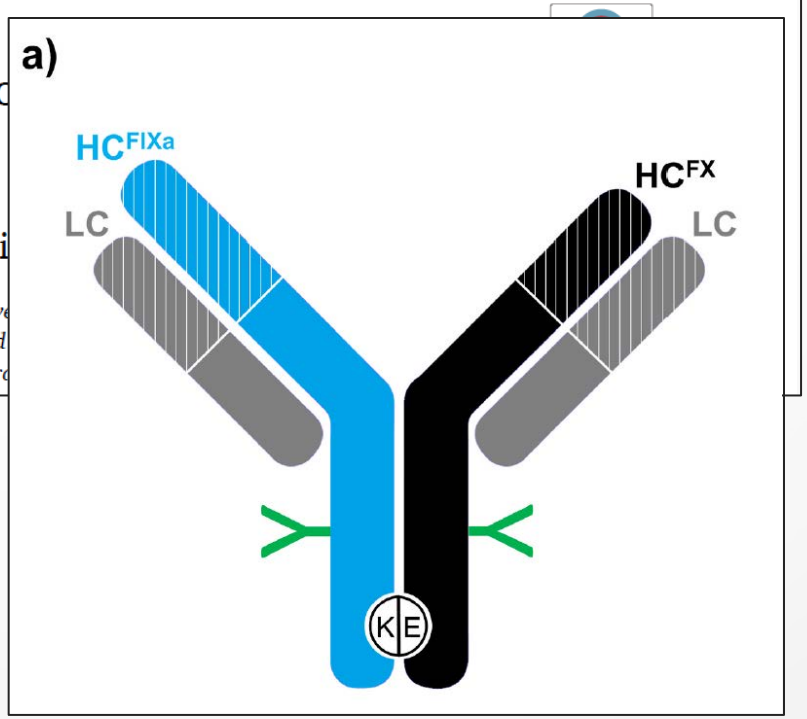
Bispecific antibody characterization by a combinatic
site-specific/chain-specific LC/MS techniques

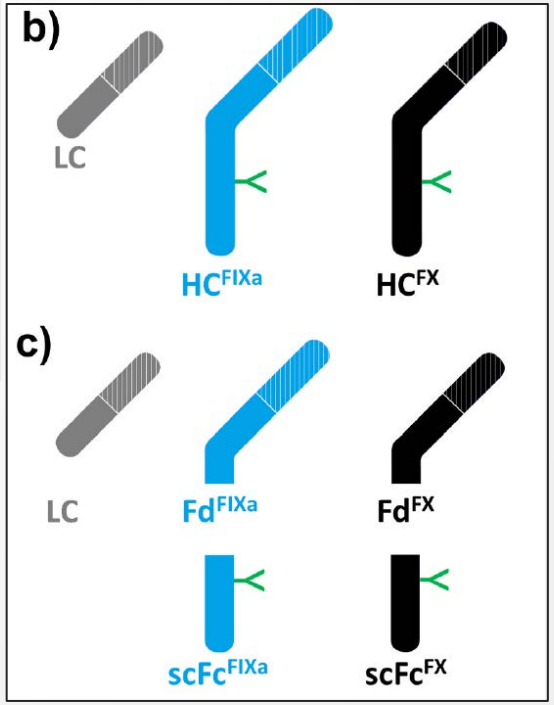
Bastiaan L. Duivelshof [a,b], Alain Beck [c], Davy Guillarme [a,b], Valenti

[a] Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, CMU-Rue Michel Serve
[b] School of Pharmaceutical Sciences, University of Geneva, CMU-Rue Michel Servet 1, 1211, Geneva 4, Switzerland
[c] IRPF - Centre d'Immunologie Pierre-Fabre (CIPF), 5 Avenue Napoléon III, BP 60497, Saint-Julien-en-Genevois, Fra

UNIVERSITÉ DE GENÈVE

V. D'Atri, D. Guillarme





- SEC-MS, CEX-MS, HILIC-MS, RPLC-MS
- Intact, Reduced, IdeS/ Reduced
- No homodimer
  - FDA BsAbs guidance 2021

New stationary phase for RP-HPLC: mAbs, BsAbs (J Chrom A 2021)

Journal of Chromatography A 1642 (2021) 462050

Contents lists available at ScienceDirect

Journal of Chromatography A

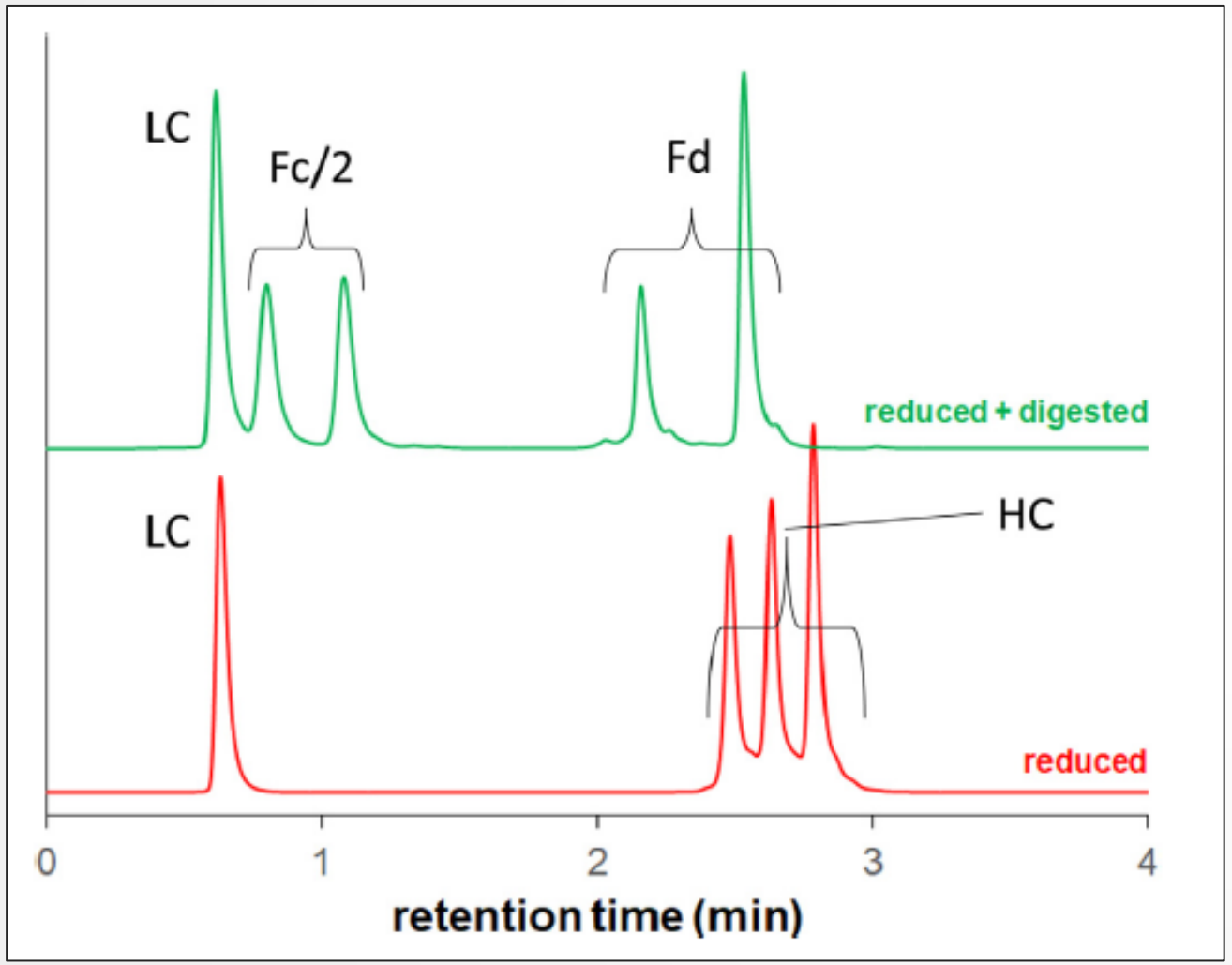
journal homepage: www.elsevier.com/locate/chroma

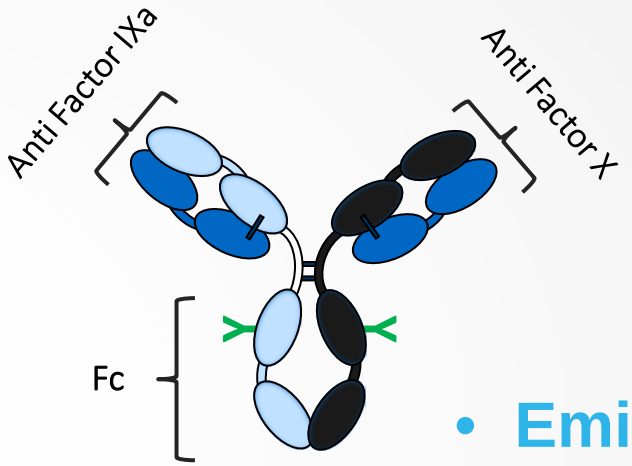
New wide-pore superficially porous stationary phases with low hydrophobicity applied for the analysis of monoclonal antibodies

Szabolcs Fekete[a,b,*], Amarande Murisier[a,b], Alain Beck[c], Jason Lawhorn[d], Harry Ritchie[d], Barry Boyes[d], Davy Guillarme[a,b]

[a] School of Pharmaceutical Sciences, University of Geneva, CMU-Rue Michel Servet 1, 1211 Geneva 4, Switzerland
[b] Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, CMU-Rue Michel Servet 1, 1211 Geneva 4, Switzerland
[c] Center of Immunology Pierre Fabre, 5 Avenue Napoléon III, BP 60497, 74160 Saint-Julien-en-Genevois, France
[d] Advanced Materials Technology, 3521 Silverside road, Suite 1-K, DE 19810, Wilmington, USA

New stationary phase for RP-HPLC: emicizumab (BsAbs, J Chrom A 2021)





- **Emicizumab**

Intact
bs-mAb
~150 kDa

Optimized fast separation of emicizumab subunits in 3 min. Column: prototype 50 ×2.1 mm, 2.7 µm 1000 ˚A ES-LH

UNIVERSITÉ DE GENÈVE

Ultra-short columns for RP-HPLC: mAbs, BsAbs (2) (Anal Chem 2021)

analytical chemistry

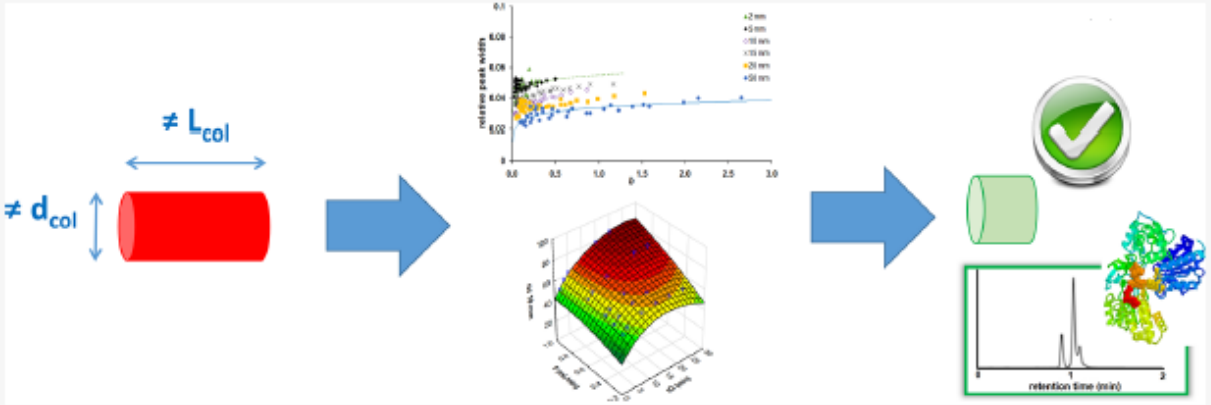
pubs.acs.org/ac Article

Use of Ultra-short Columns for Therapeutic Protein Separations, Part 2: Designing the Optimal Column Dimension for Reversed-Phase Liquid Chromatography

Szabolcs Fekete,* Amarande Murisier, Jennifer M. Nguyen, Michael J. Bolton, Jonathan Belanger, Alain Beck, Jean-Luc Veuthey, Kevin Wyndham, Matthew A. Lauber, and Davy Guillarme

Cite This: https://dx.doi.org/10.1021/acs.analchem.0c01720 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



(A) 2, 5, 10, 15 & 20 mm
(B) 50 mm

(3.2)

EDITORIAL

mAbs 3:5, 415-416; September/October 2011; © 2011 Landes Bioscience

Therapeutic Fc-fusion proteins and peptides as successful alternatives to antibodies

Alain Beck and Janice M. Reichert*

Landes Bioscience; Austin, TX USA

Fc fusion proteins & peptides (14)

1998 etanercept[PrFc] (TNFα)*

2003 alefacept[PrFc] (CD2)

2005 abatacept[PrFc][C220S,C226S,C229S,P238S] (CD80/86)*

2008 rinolacept[PrFc] (IL1) romiplostim[FcPe] (TPO)

2011 belatacept[PrFc] (CD80/86)* aflibercept[PrFc] (VEGF)

2012 ziv-aflibercept[PrFc] (VEGF)*

2014 efmoroctocog α[FVIII] eftrenonacog α[FIXFc]

2015 asfotase alfa[PrFc] (AP-TNAP) dulaglutide[PeFc][S228P,F234A,L235A] (GLP)

2016 [Benepali®/etanercept biosim, EMA/FDA]*

2019 luspatercept[PrFc] (ACVR2B)

2021 efgartigimod[Fc-5mut] (hIgG, FcR antag.)

Received: 3 July 2020 | Revised: 27 August 2020 | Accepted: 7 September 2020

DOI: 10.1002/jssc.202000765

REVIEW ARTICLE

JOURNAL OF SEPARATION SCIENCE

Therapeutic Fc-fusion proteins: Current analytical strategies

Bastiaan L. Duivelshof[1,2] | Amarande Murisier[1,2] | Julien Camperi[1,2] | Szabolcs Fekete[1,2] | Alain Beck[3] | Davy Guillarme[1,2] | Valentina D'Atri[1,2]

[1] School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland

[2] Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, Geneva, Switzerland

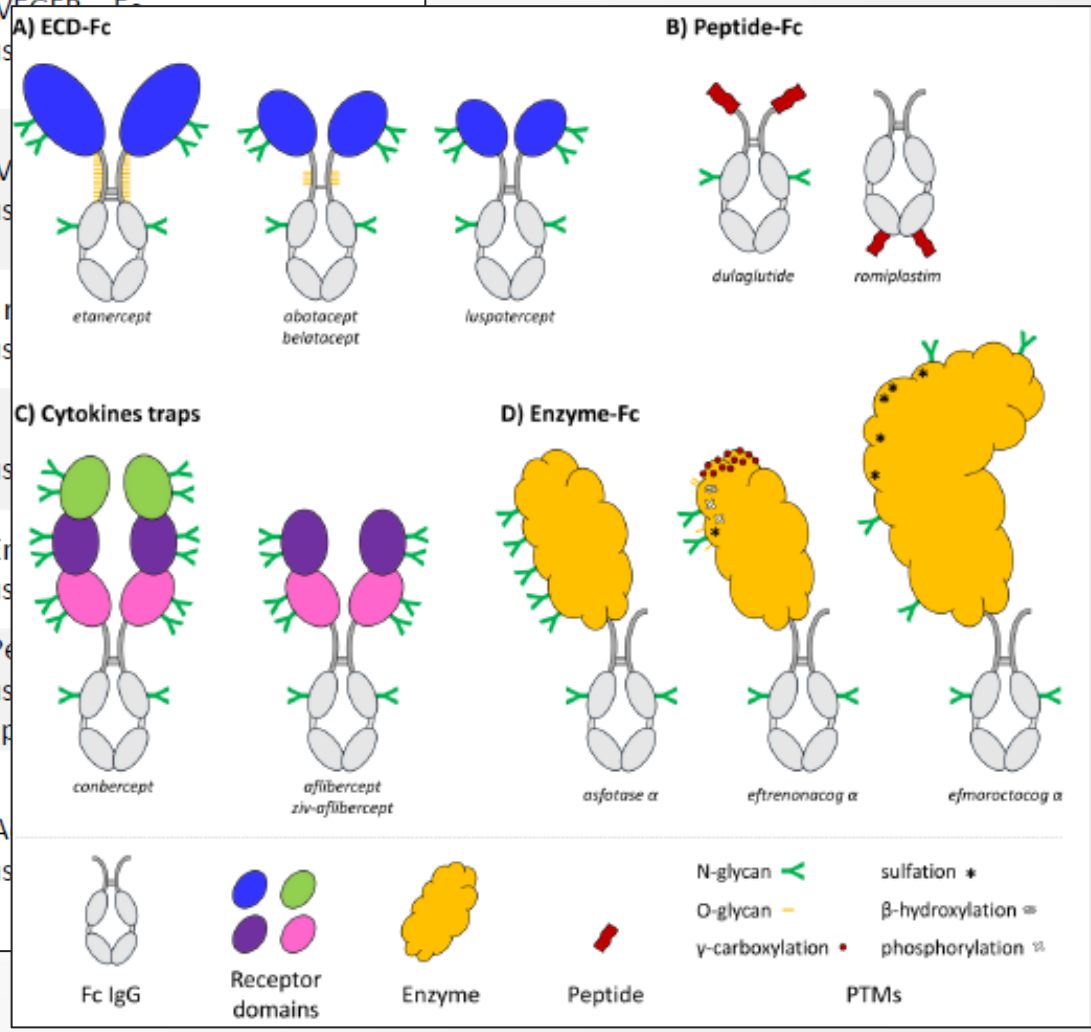
[3] IRPF - Centre d'Immunologie Pierre-Fabre (CIPF), Saint-Julien-en-Genevois, France

Correspondence
Valentina D'Atri, School of Pharmaceutical Sciences, Institute of Pharmaceutical Sciences of Western Switzerland, University

Fc-Fusion proteins represent a successful class of biopharmaceutical products, with already 13 drugs approved in the European Union and United States as well as three biosimilar versions of etanercept. Fc-Fusion products combine tailored pharmacological properties of biological ligands, together with multiple functions of the fragment crystallizable domain of immunoglobulins. There is a great diversity in terms of possible biological ligands, including the extracellular domains of natural receptors, functionally active peptides, recombinant enzymes, and genetically engineered binding constructs acting as cytokine traps. Due to their highly diverse structures, the analytical characterization of Fc-Fusion proteins is far more complex than that of monoclonal antibodies and requires the use and development of additional product-specific methods over conventional generic/platform methods. This can be explained, for example, by the presence of numerous sialic acids, leading to high diversity in terms of iso-

Therapeutic Fc-fusion proteins: current analytical strategies (JSS 2021)

1998	etanercept (Enbrel®)	ECD of the human 75 kDa (p75) tumor necrosis factor receptor (TNFR) fused to human IgG1 Fc	TNFR – Fc fusion protein	TNF-α
2003; withdrawn in 2011	alefacept (Amevive®)			
2005	abatacept (Orencia®)			
2008	rilonacept (Arcalyst®)			
2008	romiplostim (Nplate®)			
2011	belatacept (Nulojix®)			
2011	aflibercept (Eylea®)			
2012	ziv-aflibercept (Zaltrap®)	ECDs of human vascular endothelial growth factor (VEGF) receptor 1 (domain 2) and receptor 2 (domain 3) fused to human IgG1 Fc		
2013 (by CFDA)	conbercept (Lumitin®)	ECDs of human vascular endothelial growth factor (VEGF) receptor 1 (domain 2) and receptor 2 (domains 3 and 4) fused to human IgG1 Fc		
2014	efmoroctocog α (Elocta®)	Single molecule of recombinant Factor VIII (rFVIII) fused to human IgG1 Fc		
2014	eftrenonacog α (Alprolix®)	Single molecule of recombinant Factor IX (rFIX) fused to human IgG1 Fc		
2015	asfotase α (Strensiq®)	Catalytic domain of tissue-nonspecific alkaline phosphatase (TNSALP) fused to the human IgG1 Fc		
2015	dulaglutide (Trulicity®)	Dipeptidyl peptidase-IV-protected glucagon-like peptide (GLP-1) fused to human IgG4 Fc		
2019	luspatercept (Reblozyl®)	Modified ECD of activin receptor type IIB (actRIIb) fused to human IgG1 Fc		



- **V. D'Atri et al**
- **Duivelshof B, Beck A, Guillarme D, D'Atri V et al, 2021**

Etanercept N & O-glycans: HILIC-MS, middle-up (2019)

analytical chemistry

Article

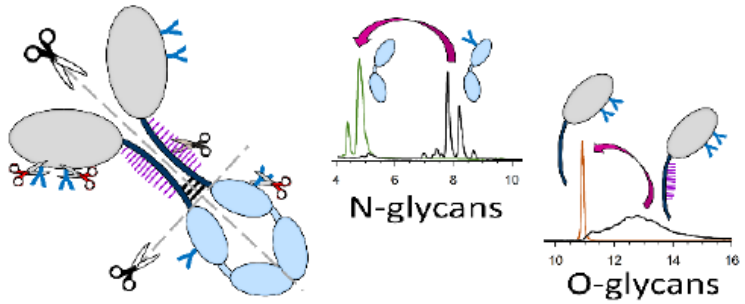
Cite This: *Anal. Chem.* 2019, 91, 873−880

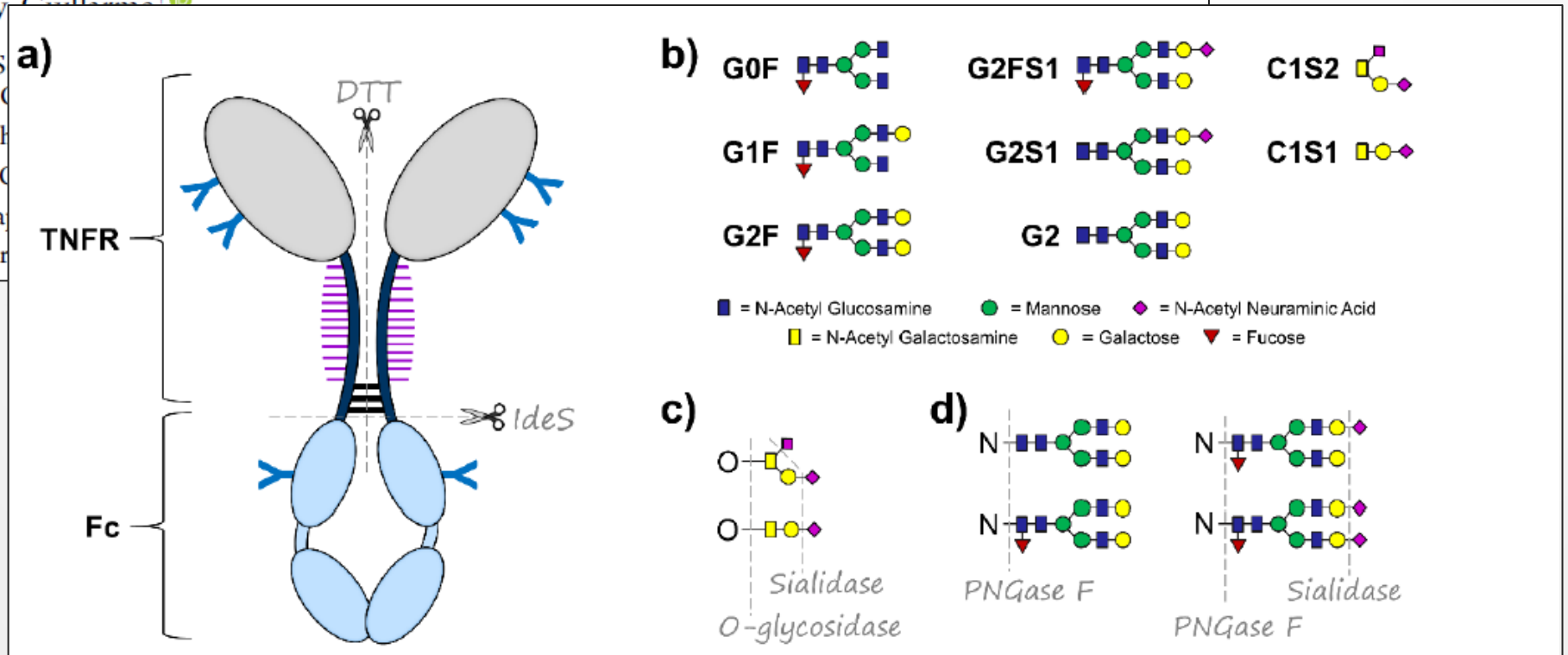
pubs.acs.org/ac

Orthogonal Middle-up Approaches for Characterization of the Glycan Heterogeneity of Etanercept by Hydrophilic Interaction Chromatography Coupled to High-Resolution Mass Spectrometry

Valentina D'Atri,[*,†,#] Lucie Nováková,[‡,#] Szabolcs Fekete,[†] Dwight Stoll,[§] Matthew Lauber,[∥] Alain Beck,[⊥] and Davy Guillarme[†]

[†]Section of Pharmaceutical S
Rue Michel Servet 1, 1211 C
[‡]Department of Analytical Ch
[§]Department of Chemistry, C
[∥]Waters Corporation, 34 Ma
[⊥]Center of Immunology Pier





Therapeutic Abs: Meth. &Prot. (Gunnar Houen, MiMB 2022)

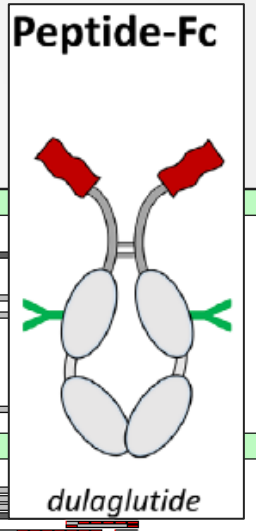
Check for updates

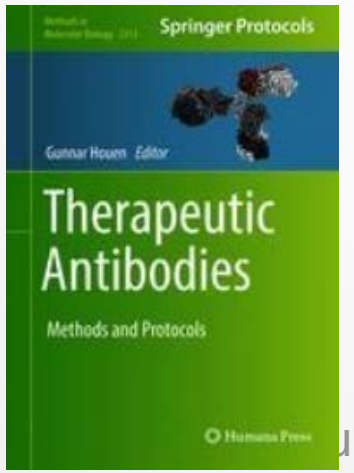
Chapter 12

Use of PASEF for Accelerated Protein Sequence Confirmation and *De Novo* Sequencing with High Data Quality

Detlev Suckau, Waltraud Evers, Eckhard Belau, Stuart Pengelley, Anja Resemann, Wilfred Tang, K. Ilker Sen, Elsa Wagner, Olivier Colas, and Alain Beck

- Parallel Accumulation and Serial Fragmentation (PASEF)
- Dulaglutide





BRUKER

Pierre Fabre

Public

(VI) EDA-Fc fusion protein, XLHED (Dec 2020)

The EspeRare Foundation and Pierre Fabre join forces to develop and market a pioneering treatment for XLHED, a dermatologic-related rare genetic disease that requires prenatal therapeutic intervention

Pierre Fabre
The EspeRare Foundation
2020-12-14 09:00 425

GENEVA and CASTRES, France, Dec. 14, 2020 /PRNewswire/ -- The EspeRare Foundation and the Pierre Fabre group announced today that they have entered into a license and development collaboration agreement for the development and commercialization of ER-004, a prenatal treatment for XLHED (X-linked Hypohidrotic Ectodermal Dysplasia), a rare, debilitating congenital disease. The next clinical study is expected to start in 2021 and will aim at qualifying and registering what may become the first approved treatment for XLHED by 2026.

According to the terms of the agreement, EspeRare and the Pierre Fabre Group will pool their respective expertise together in order to co-develop ER-004. The Pierre Fabre group will be granted exclusive worldwide rights for the development, manufacturing and commercialization of ER-004.

Pierre Fabre Selects AGC Biologics as CDMO to manufacture the orphan drug ER-004

AGC Biologics

News provided by

AGC Biologics

Dec 21, 2020, 10:00 ET

SEATTLE, Dec. 21, 2020 /PRNewswire/ -- AGC Biologics, a leading global Biopharmaceutical Contract Development and Manufacturing Organization (CDMO), announced its partnership with Laboratoire Pierre Fabre to manufacture ER-004 – an intra-amniotic drug that will pioneer the treatment of a rare and debilitating genetic disorder. AGC Biologics will manufacture GMP material for the next stage of clinical trial.

"We are very pleased that Pierre Fabre has entrusted us with the manufacture of this product," says AGC Biologics Chief Business Officer, Mark Womack. "We are really looking forward to seeing this treatment go to the market."

Pierre Fabre has entered into a development and license agreement with the Switzerland-based EspeRare Foundation on ER-004, a fusion protein involved in X-Linked Hypohidrotic Ectodermal Dysplasia (XLHED), a rare genetic disorder affecting ectodermal structures including sweat glands, respiratory glands, skin, hair, and teeth. Clinical manifestations of XLHED are severe and can include severe episodes of hyperthermia, heat intolerance, and an increased risk of serious respiratory tract infections. Delivered through intra-amniotic injections during the late stage of pregnancy, ER-004 shows significant potential in inducing the growth of affected ectodermal structures, resulting in normalized sweat gland function.

(3.3) ADC Landscape (Pharmaceuticals 2011/2022)

pharmaceuticals

MDPI

Review

Antibody–Drug Conjugates: The Last Decade

Nicolas Joubert [1,*], Alain Beck [2], Charles Dumontet [3,4] and Caroline Denevault-Sab

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* Correspondence: nicolas.joubert@univ-tours.fr

Received: 17 August 2020; Accepted: 10 September 2020; Published: 14 September 2020

Abstract: An armed antibody (antibody–drug conjugate or ADC) is a vectorized
which results from the grafting of a cytotoxic agent onto a monoclonal antibody v
constructed spacer arm. ADCs have made considerable progress in 10 years. Wh
gemtuzumab ozogamicin (Mylotarg®) was used clinically, in 2020, 9 Food and Drug
(FDA)-approved ADCs are available, and more than 80 others are in active clini
review will focus on FDA-approved and late-stage ADCs, their limitations includi
and associated resistance mechanisms, as well as new emerging strategies to addr
and attempt to widen their therapeutic window. Finally, we will discuss their co

- 11 ADC vs 5 BsAbs FDA approv.
  - + 1 China
- Mature class of oncology drugs

Table 1. Antibody–drug conjugates (ADCs) approved by the Food and Drug Administration (FDA), in advanced clinical trials (Phase III or pivotal phase II) or recently stopped.

Company	ADC (Cytotoxic)	Isotype and Target	Indication/Approval Date (Trade Name)/Clinical Status
Pfizer	gemtuzumab ozogamicin (CAL)	IgG4 CD33	2000–2010/2017 AML (Mylotarg®)
Seattle Genetics	brentuximab vedotin (AUR)	IgG1 CD30	2011 ALCL and Hodgkin lymphoma (Adcetris®)
Roche	trastuzumab emtansine (MAY)	IgG1 HER2+	2013 metastatic HER2+++ breast cancer (Kadcyla®) **
Pfizer	inotuzumab ozogamicin (CAL)	IgG4 CD22	2017 ALL and CLL (Besponsa®)
Roche	polatuzumab vedotin (AUR)	IgG1 CD79b	2019 DLBCL (Polivy®)
Seattle Genetics	enfortumab vedotin (AUR)	IgG1 Nectin 4	2019 urothelial cancer (Padcev®) **
Daiichi Sankyo	trastuzumab deruxtecan (EXA)	IgG1 HER2+	2019 metastatic HER2+++ breast cancer (Enhertu®) **
Immunomedics	sacituzumab govitecan (IRI)	IgG1 TROP-2	2020, metastatic TNBC (Trodelvy®) **
GSK	belantamab mafodotin (AUR, MMAF)	IgG1afuc BCMA	2020, multiple myeloma (Blenrep®)

Open access: https://www.mdpi.com/1424-8247/13/9/245/pdf

ADCs structural characterization reviews

EXPERT REVIEW OF PROTEOMICS, 2016
http://dx.doi.org/10.1586/14789450.2016.1132167

(2016)

Taylor & Francis
Taylor & Francis Group

REVIEW

Cutting-edge mass spectrometry methods for the multi-level structural characterizatio

Alain Beck[a], Guillaume
Bussat[a], Olivier Colas[a],

[a]Centre d'Immunologie Pi
Analytical Sciences Depart
Strasbourg, France

EXPERT REVIEW OF PROTEOMICS
https://doi.org/10.1080/14789450.2019.1578215

(2019)

Taylor & Francis
Taylor & Francis Group

Check for updates

REVIEW

Cutting-edge multi-level analytical and structural characterization of antibody-drug conjugates: present and future

Alain Beck[a], Valentina D'Atri[b], Anthony Ehkirch[c], Szabolcs Fekete[b], Oscar Hernandez-Alba[c], Rabah Gahoual[d], Emmanuel Leize-Wagner[d], Yannis François[d], Davy Guillarme[a] and Sarah Cianférani[c]

[a]IRPF - Centre d'Immunologie Pierre-Fabre (CIPF), Saint-Julien-en-Genevois, France; [b]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CMU, Geneva, Switzerland; [c]Laboratoire de Spectrométrie de Masse BioOrganique, IPHC UMR 7178, Université de Strasbourg, CNRS, Strasbourg, France; [d]Laboratoire de Spectrométrie de Masse des Interactions et des Systèmes (LSMIS), UMR 7140, Université de Strasbourg, CNRS, Strasbourg, France

GlyGLICK ADCs (Genovis): HILIC-MS (Anal Chem 2020)

Glycan-mediated technology for obtaining homogeneous site-specific conjugated antibody-drug conjugates: synthesis and analytical characterization by using complementary middle-up LC/HRMS analysis

Bastiaan L. Duivelshof,† Evolène Deslignière,‡ Oscar Hernande
Toftevall,‡ Jonathan Sjögren,‡ Sarah Cianferani,‡ Alain Beck,§ Davy

†Institute of Pharmaceutical Sciences of Western Switzerland, University of Genev
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‡Laboratoire de Spectrométrie de Masse BioOrganique, IPHC UMR 7178, Un
France.

‡Genovis AB, Box 790, SE-220 07 Lund, Sweden.

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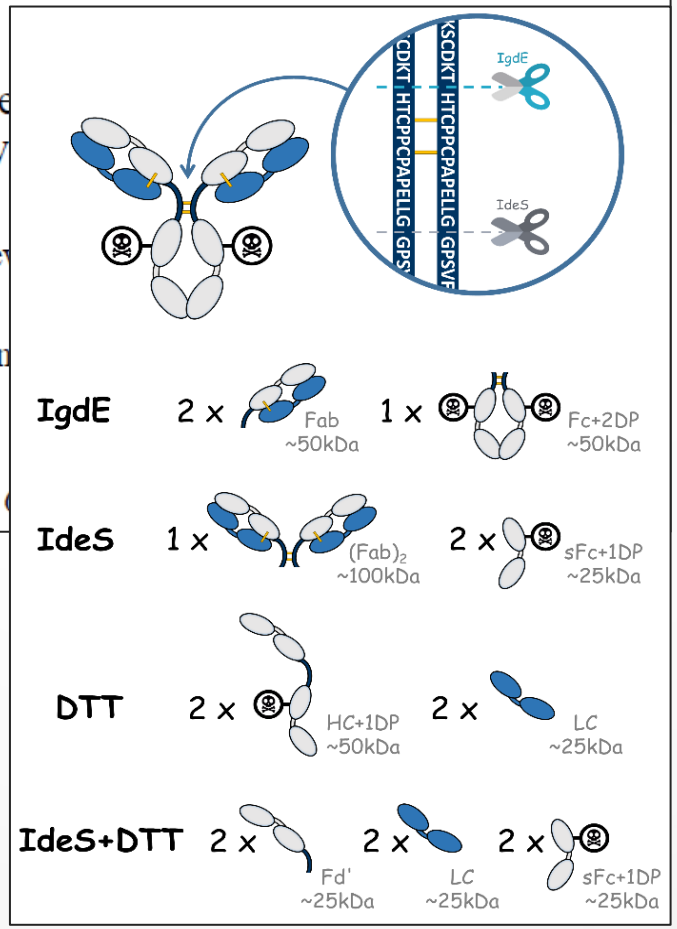
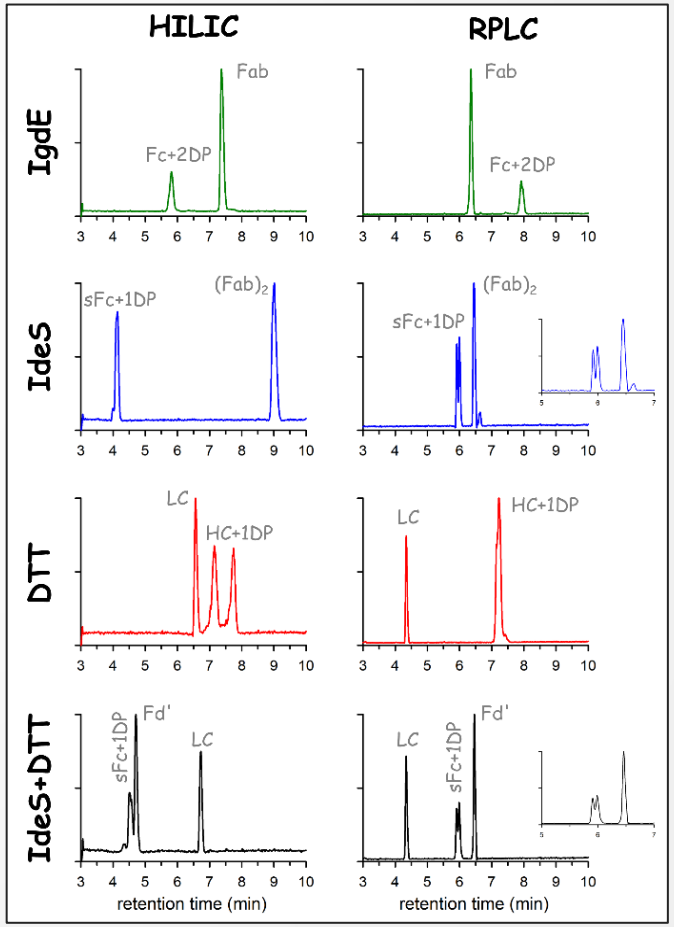
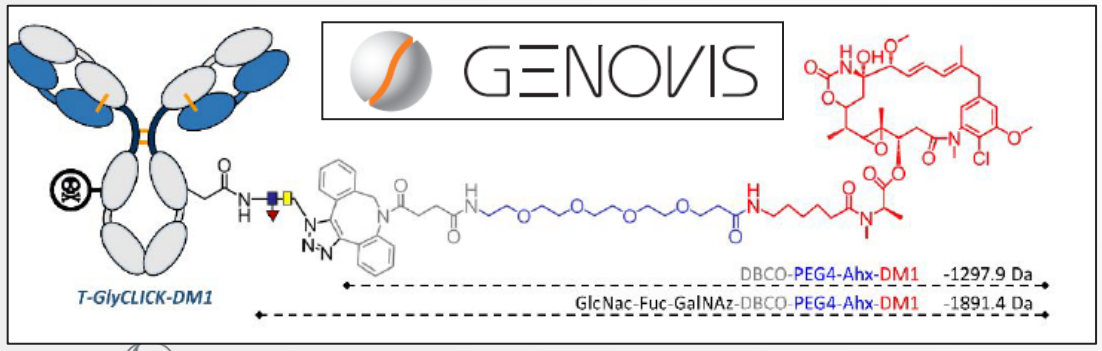
V. D'Atri, D. Guillarme & coll.







Native MS and IMMS for mAbs and ADCs (2021)

pharmaceuticals

MDPI

Article

State-of-the-Art Native Mass Spectrometry and Ion Mobility Methods to Monitor Homogeneous Site-Specific Antibody-Drug Conjugates Synthesis

Evolène Deslignière [1,2], Anthony Ehkirch [1,2], Bastiaan L. Duivelshof [3,4], Hanna Toftevall [5], Jonathan Sjögren [5], Davy Guillarme [3,4], Valentina D'Atri [3,4], Alain Beck [6], Oscar Hernandez-Alba [1,2] and Sarah Cianférani [1,2,*]

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ahernandez@unist
2 Infrastructure Nati
3 School of Pharmace
Bastiaan.Duivelsho
4 Institute of Pharma
1211 Geneva, Switz
5 Genovis AB, SE-220
6 IRPF—Centre d'Im

Laboratoire de Spectrométrie de Masse Bio-Organique

- E. Deslignière
- S. Cianférani

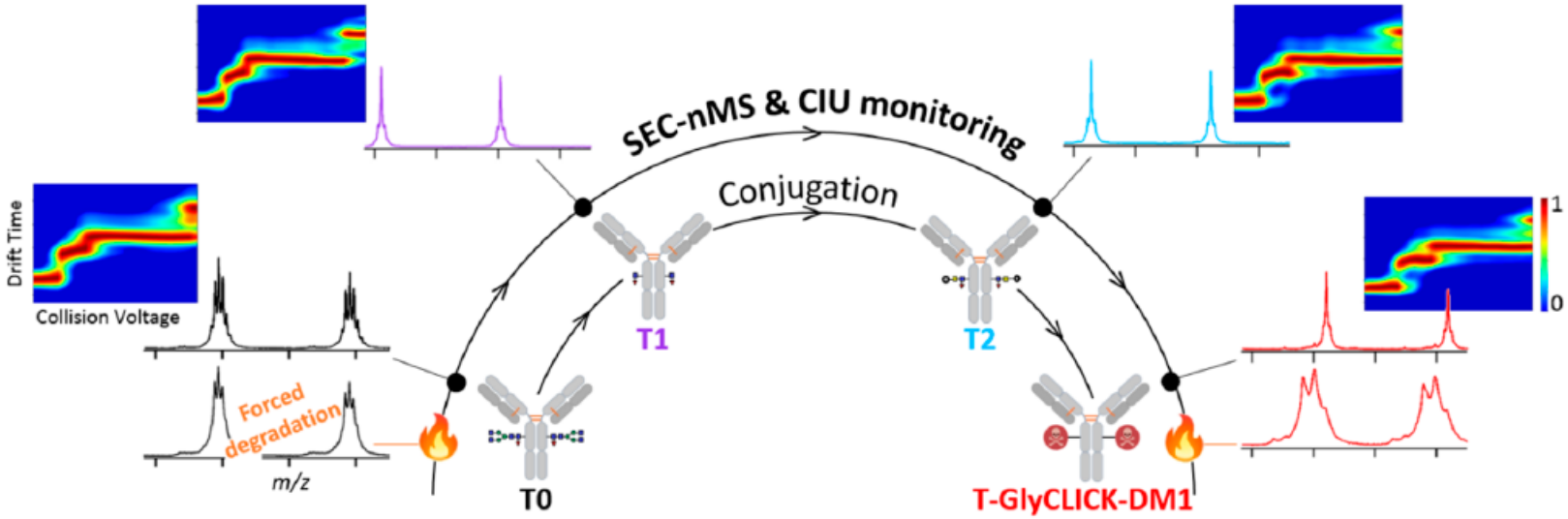
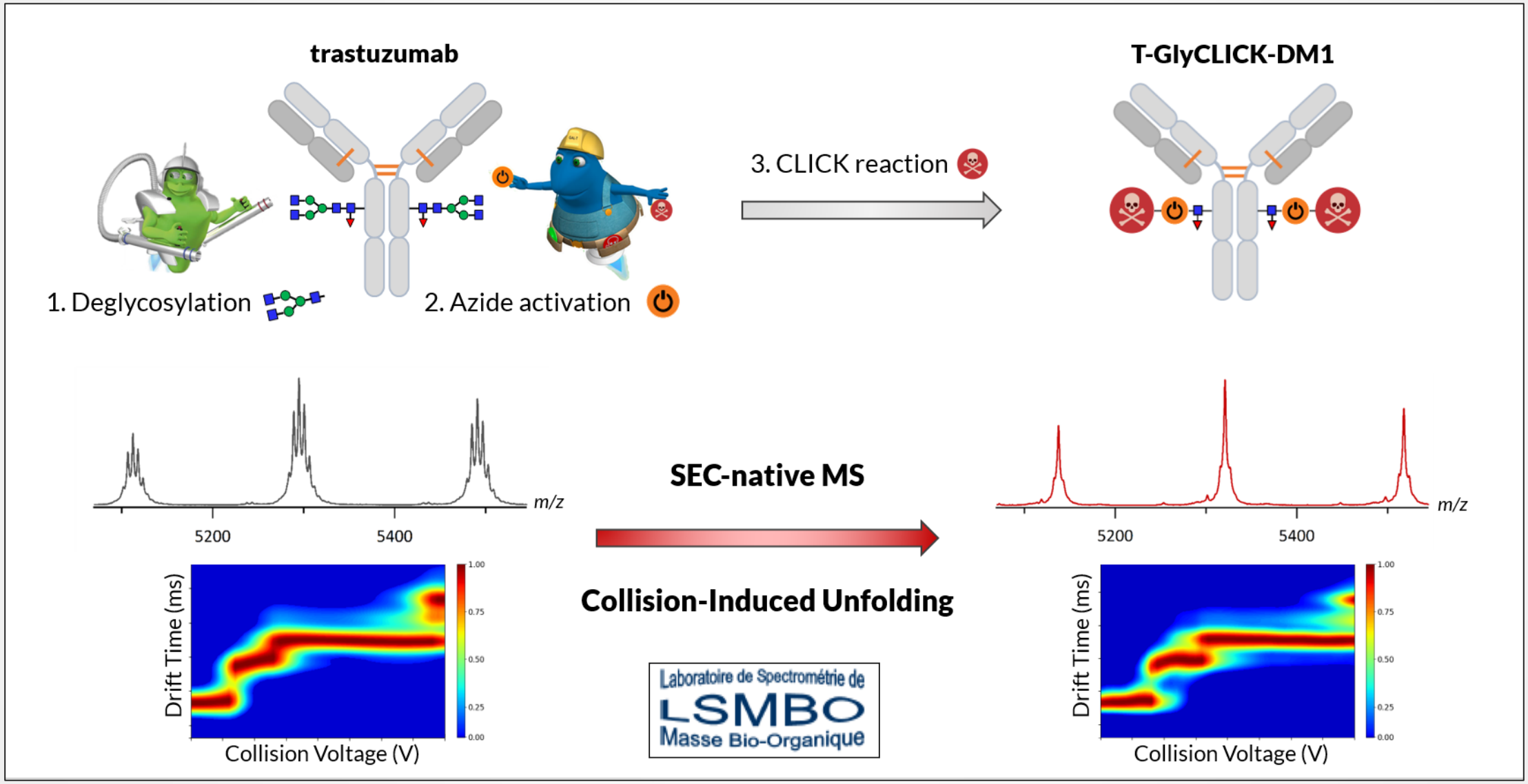
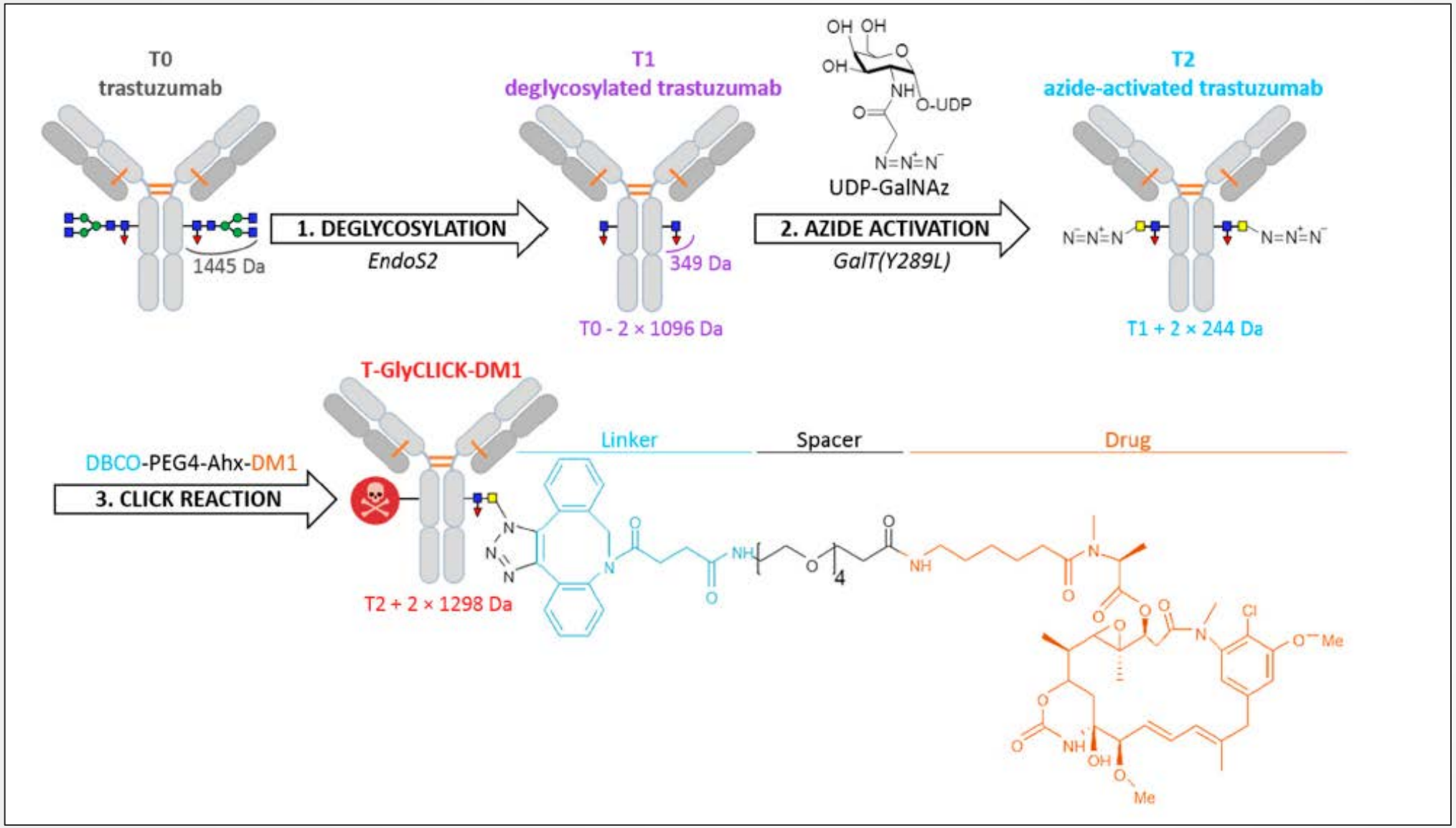


Figure 2. Analytical workflow used to monitor the conjugation of T-GlyCLICK-DM1.

GlyGLICK ADCs (Genovis): Native MS (2021)

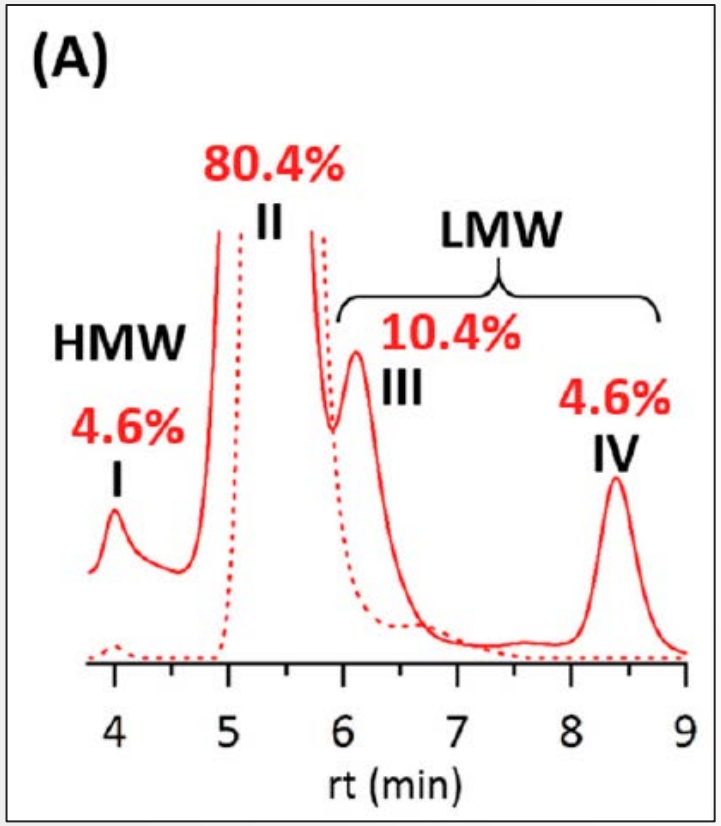


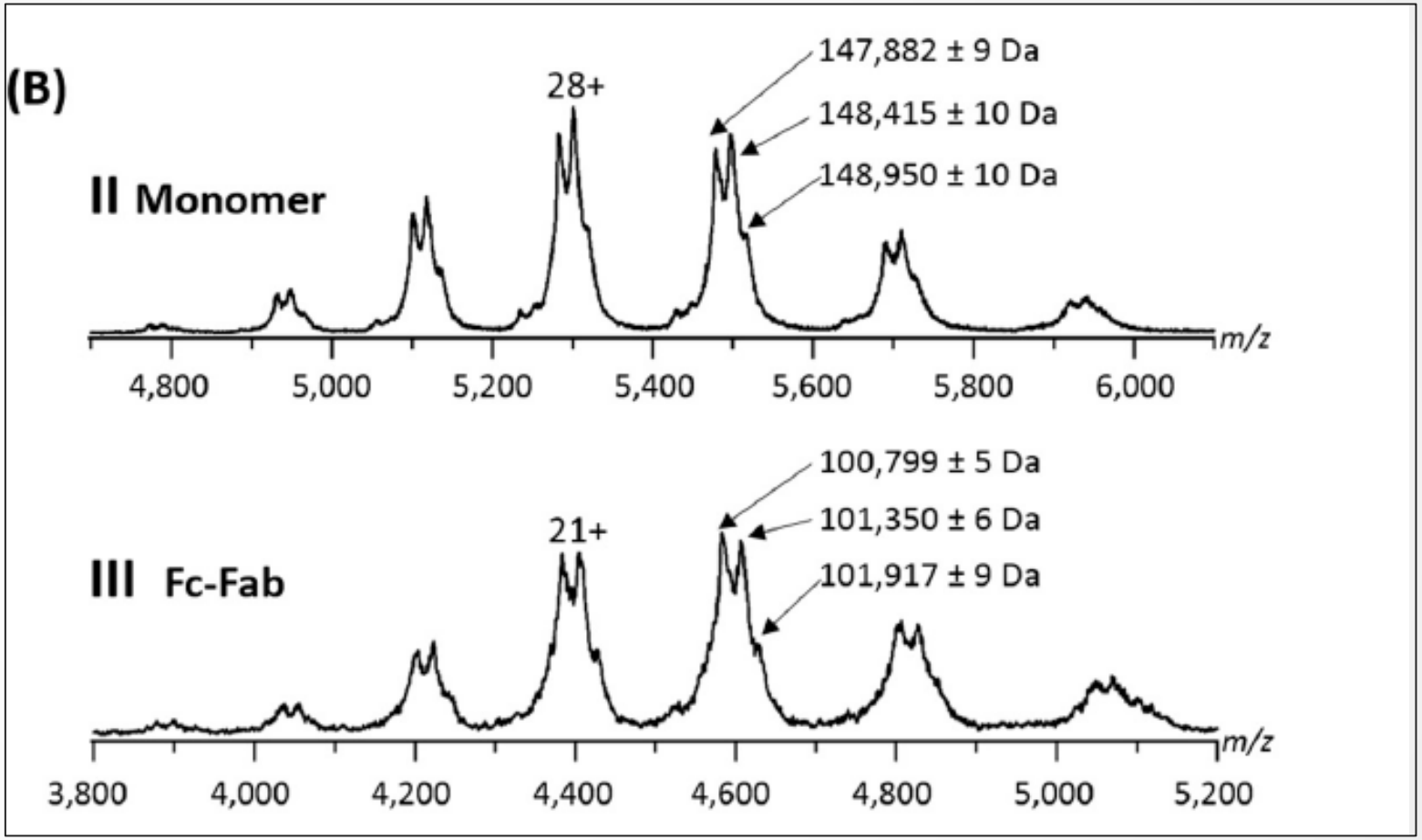
GlyGLICK ADCs (Genovis): Native MS (2021)



- **J. Sjögren**

GlyGLICK ADCs (Genovis): Native MS (2021)

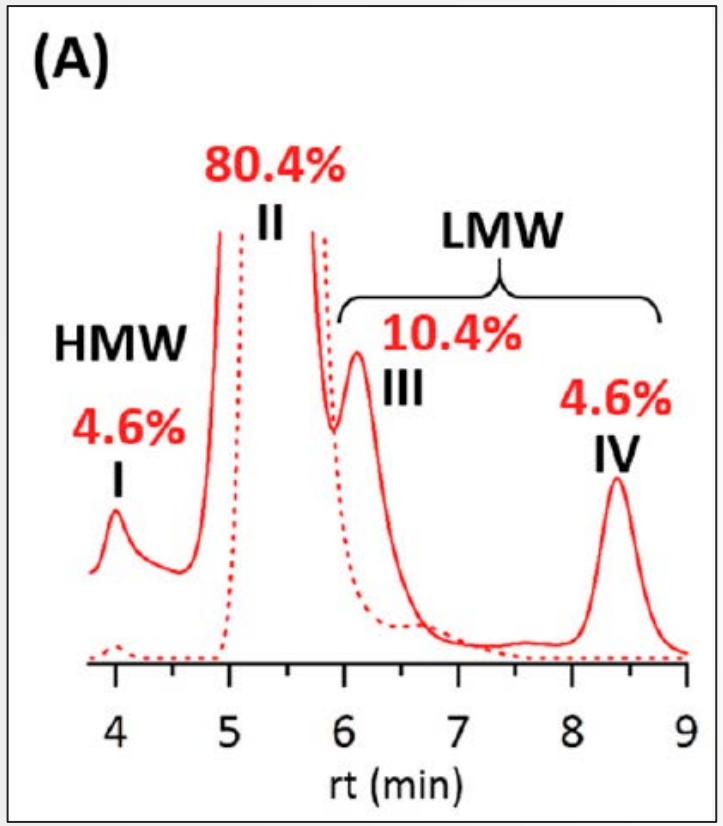


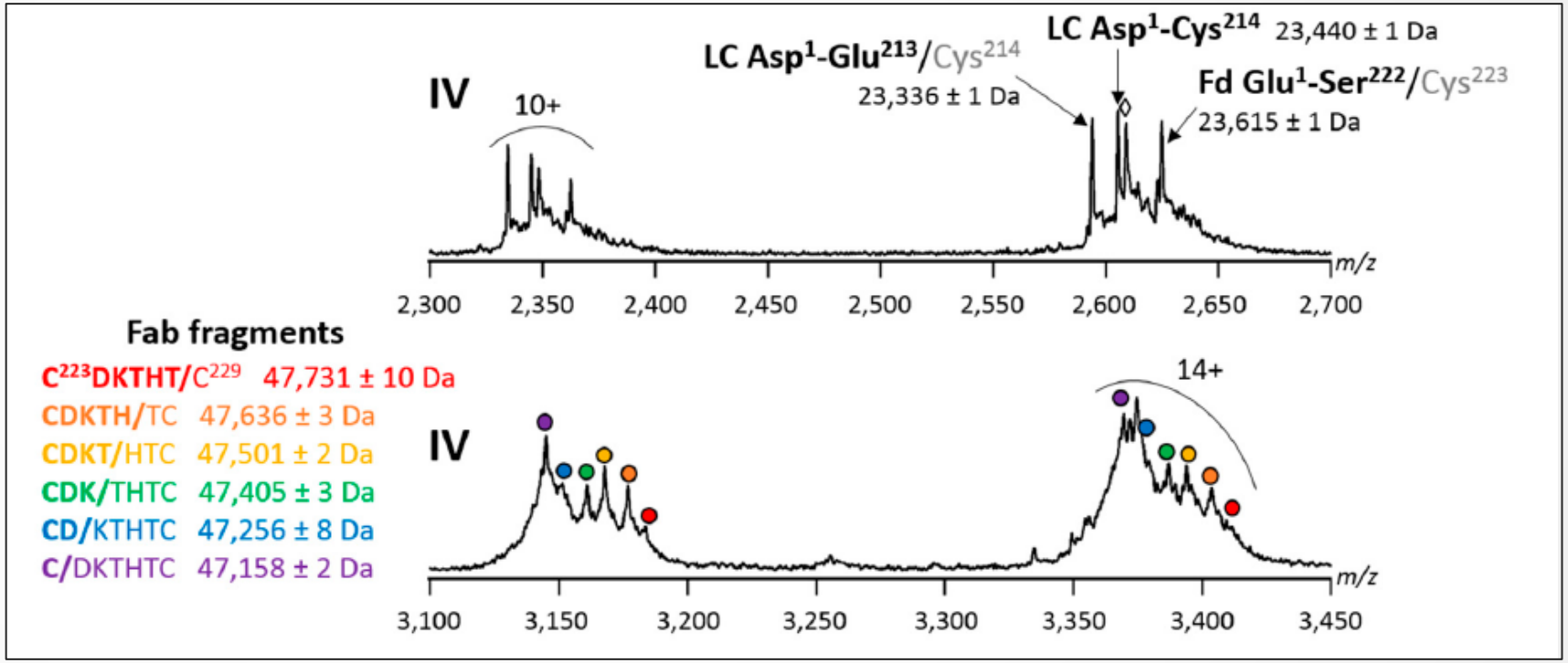


Size variants (SEC-MS): similar

- **HMWS and**
- **LMWS (Fab-Fc 100 KDa; Fab 50 KDa) species as for naked antibodies**

GlyGLICK ADCs (Genovis): Native MS (2021)





Size variants (SEC-MS): similar

- **HMWS and**
- **LMWS (Fab-Fc 100 KDa; Fab 50 KDa) species as for naked antibodies**

Ultra-short columns for RP-HPLC: mAbs & ADCs (1) (Anal Chem 2021)

analytical chemistry

pubs.acs.org/ac

Use of Ultrashort Columns for Therapeutic Protein Se
1: Theoretical Considerations and Proof of Concept

Szabolcs Fekete,* Balázs Bobály, Jennifer M. Nguyen, Alain Beck, Jean-Luc Veu
Matthew A. Lauber, and Davy Guillarme

Cite This: *Anal. Chem.* 2021, 93, 1277–1284

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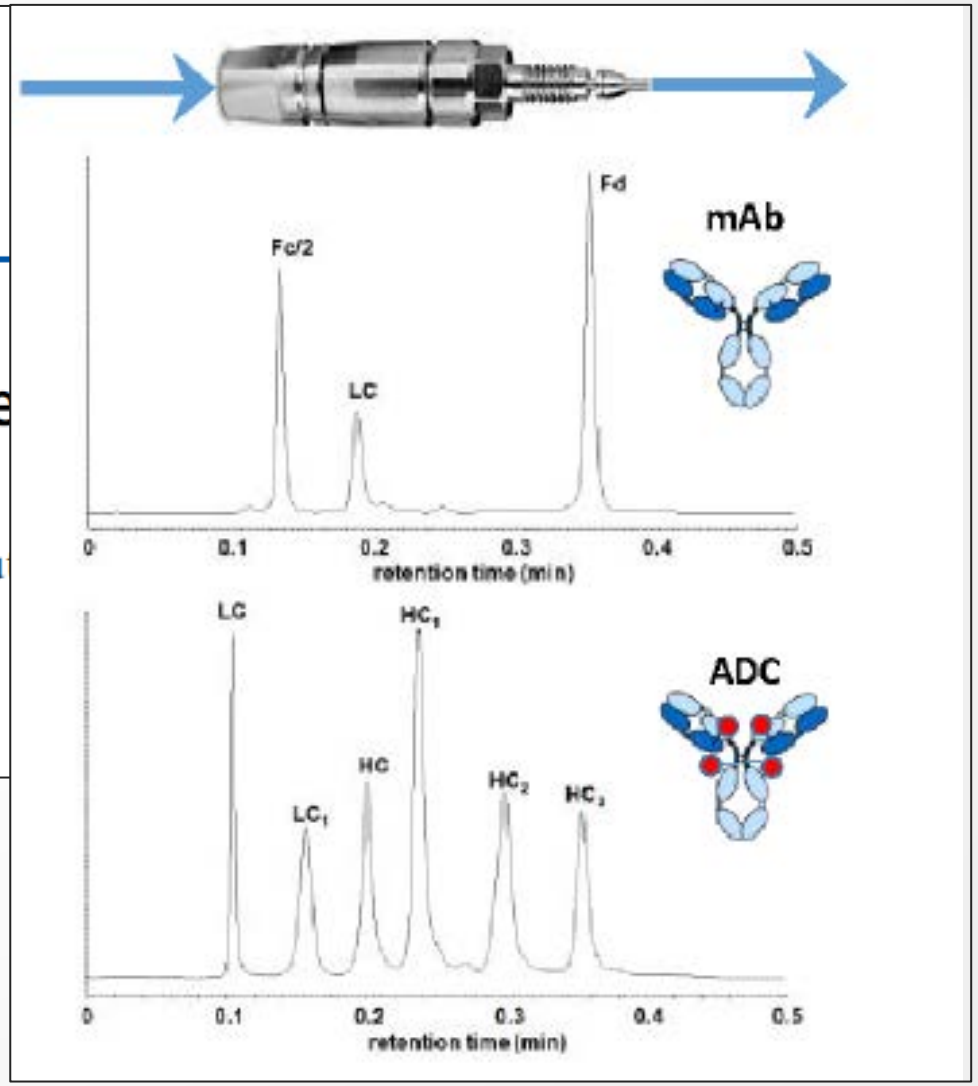
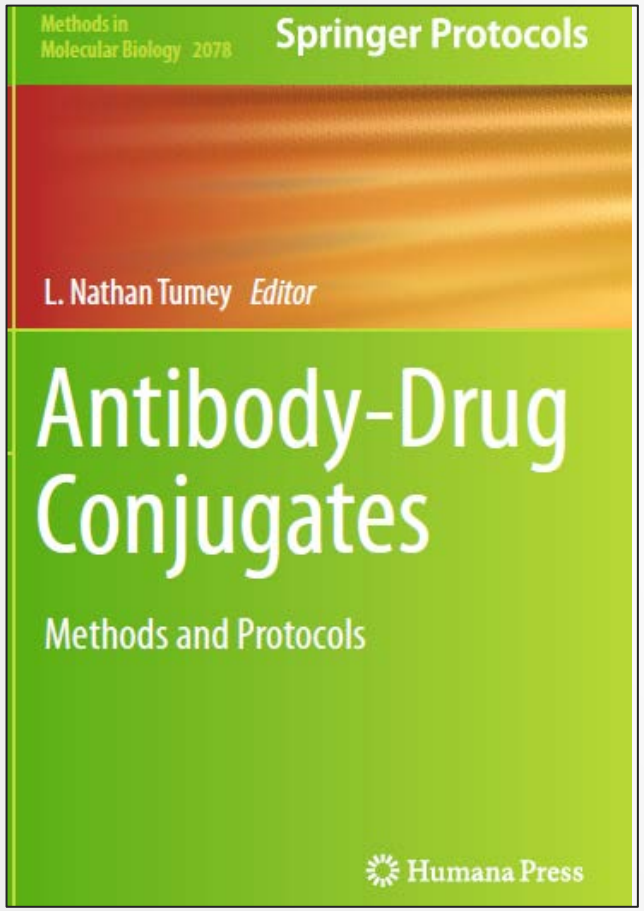


- **Brentuximab vedotin**
- **< 1 min elution**

ADCs: Methods & Protocols (N. Thumey, MiMB 2020)



Check for updates

Chapter 12

Drug Lo
or IdeS

Elsa Wag
Sabine H

Check for updates

Chapter 13

Analysis o

Oscar Herna
and Sarah C

Check for updates

Chapter 18

Characterization of the Primary Structure of Cysteine-Linked Antibody-Drug Conjugates Using Capillary Electrophoresis with Mass Spectrometry

Josiane Saadé, Rabah Gahoual, Alain Beck, Emmanuelle Leize-Wagner, and Yannis-Nicolas François

(VII) Telisotuzumab vedotin (cMet, PhIII, NSCLC) - 2018

Cancer Therapy: Preclinical

Clinical Cancer Research

ABBV-399, a c-Met Antibody–Drug Conjugate that Targets Both *MET*–Amplified and c-Met–Overexpressing Tumors, Irr*MET* Pathway Dependence

Jieyi Wang[1], Mark G. Anderson[1], Anatol Oleks
Lora Tucker[1], Qian Zhang[1], Edward K. Han[1], J
Edward B. Reilly[1]

NCT02099058 (PhI)
NCT03539536 (PhII, NSCLC)

abbvie

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

First-in-Human Phase I, Dose-Escalation and -Expansion Study of Telisotuzumab Vedotin, an Antibody–Drug Conjugate Targeting c-Met, in Patients With Advanced Solid Tumors

John H. Strickler, Colin D. Weekes, John Nemunaitis, Ramesh K. Ramanathan, Rebecca S. Heist, Daniel Morgensztern, Eric Angevin, Todd M. Bauer, Huibin Yue, Monica Motwani, Apurvasena Parikh, Edward B. Reilly, Daniel Afar, Louie Naumovski, and Karen Kelly

Author affiliations and support information (if applicable) appear at the end of this article.

Published at jco.org on October 4, 2018.

Clinical trial information: NCT02099058.

Corresponding author: John H. Strickler,

A B S T R A C T

Purpose

This first-in-human study evaluated telisotuzumab vedotin (Teliso-V), formerly called ABBV-399, an antibody–drug conjugate of the anti–c-Met monoclonal antibody ABT-700 and monomethyl auristatin E.

(VII) Telisotuzumab vedotin: Non-Small Cell Lung Cancer, PhIII (FDA - Jan 5, 2022)

FDA Grants Breakthrough Therapy Designation to Teliso-V for Treatment of NSCLC

January 5, 2022
Ashley Gallagher, Assistant Editor

Telisotuzumab vedotin (Teliso-V, AbbVie) is an investigational antibody-drug conjugate that targets c-Met, a receptor tyrosine kinase that is overexpressed in tumors.

The FDA has granted breakthrough therapy designation (BTD) to investigational telisotuzumab vedotin (Teliso-V, AbbVie) for the treatment of individuals with advanced/metastatic epidermal growth factor receptor (EGFR) wild type, nonsquamous non–small-cell lung cancer (NSCLC), with high levels of c-Met overexpression whose disease has progressed on or after platinum-based therapy.

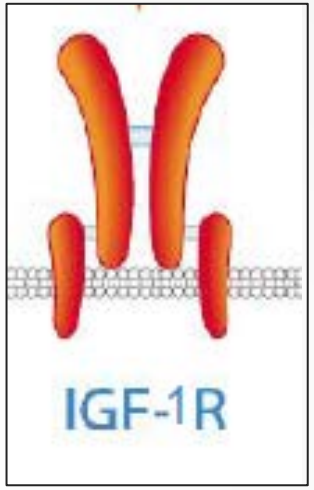
(VIII) IGFR-1 ADC: W0101 (MCT 2020)
lonigutamab ugodotin (W0101)

Pierre Fabre

MOLECULAR CANCER THERAPEUTICS | LARGE MOLECULE THERAPEUTICS

Efficacy of the Antibody–Drug Conjugate W0101 in Preclinical Models of IGF-1 Receptor Overexpressing Solid Tumors

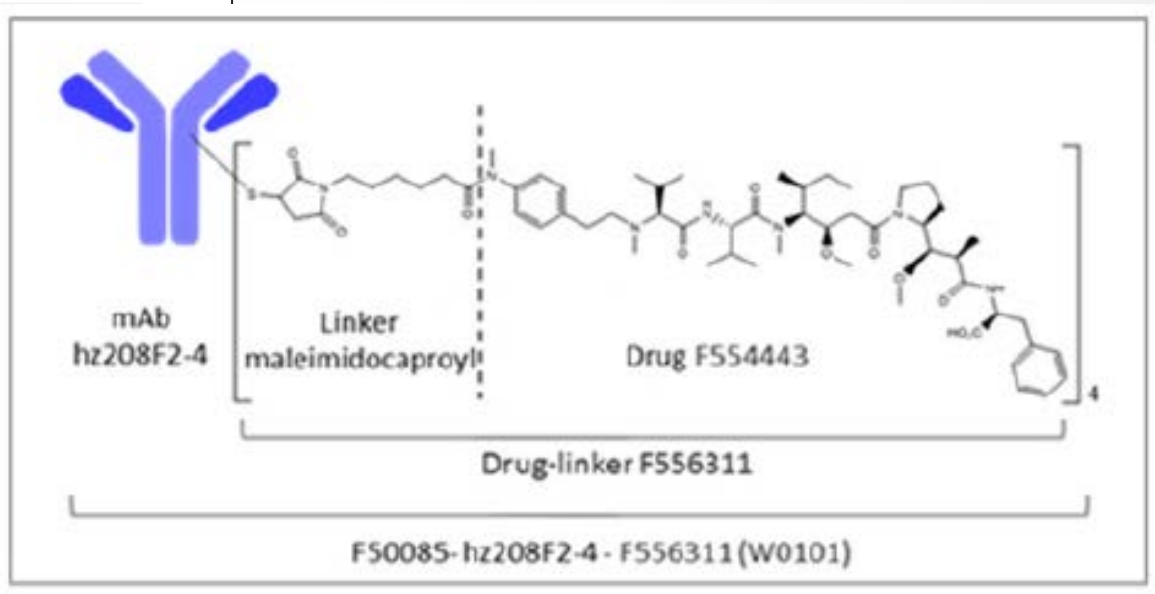
Barbara Akla[1], Matthieu Broussas[1], Noureddine Loukili[1], Alain Robert[1], Charlotte Beau-Larvor[1], Martine Malissard[1], Nicolas Boute[1], Thierry Champion[1], Jean-Francois Haeuw[1], Alain Beck[1], Michel Perez[2], Cyrille Dreyfus[1], Mariya Pavlyuk[2], Eric Chetaille[2], and Nathalie Corvaia[1]



PhI/II trial
NCT03316638
Solid tumors

ABSTRACT

The insulin-like growth factor type 1 receptor (IGF-1R) is important in tumorigenesis, and its overexpression occurs in numerous tumor tissues. To date, therapeutic approaches based on mAbs and tyrosine kinase inhibitors targeting IGF-1R have only shown clinical benefit in specific patient populations. We report a unique IGF-1R–targeted antibody–drug conjugate (ADC), W0101, designed to deliver a highly potent cytotoxic auristatin derivative selectively to IGF-1R overexpressing tumor cells. The mAb (hz208F2-4) used to prepare the ADC was selected for its specific binding properties to IGF-1R compared with the insulin receptor, and for its internalization properties. Conjugation of a novel auristatin derivative drug linker to hz208F2-4 did not alter its binding and internalization properties. W0101 induced receptor-dependent cell cytotox when applied to various cell lines overexpressing IG did not affect normal cells. Efficacy studies were c several mouse models expressing different levels o determine the sensitivity of the tumors to W0 induced potent tumor regression in certain mo Interestingly, the potency of W0101 correlated witl sion level of IGF-1R evaluated by IHC. In an M cancer model with high-level IGF-1R expression, a tion of W0101 3 mg/kg led to strong inhibition of tu W0101 provides a potential new therapeutic option overexpressing IGF-1R. A first-in-human trial o currently ongoing to address clinical safety.



R&D PHARMA

Pierre Fabre

(4) Take home messages:

Covid-19, networking, open research, white papers

- Brian Kelley, Nature Biotech 2020

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comment

Developing therapeutic monoclonal antibodies at pandemic pace

The time from discovery to proof-of-concept trials could be reduced to 5–6 months from a traditional timeline of 10–12 months.

Brian Kelley

Outbreaks of emerging infectious diseases have become increasingly common in recent decades. Epidemics have spread across the globe, including AIDS, H1N1 influenza and most recently coronavirus disease (COVID-19). In the face of a pandemic infectious disease outbreak, new approaches should be explored to enable the most rapid evaluation of antibodies for passive immunization or treatment. The fastest timeline from discovery to clinical evaluation of novel recombinant antibodies for medical use has been a focus of the biopharmaceutical industry for decades. For potentially life-saving therapies, the benefits of the earliest clinic testing should translate to accelerated pivotal trial testing and maximal patient benefit. Process and advances and the acceptance of business (but not product quality or patient safety) risks offers a further acceleration for clinical trials. Rapid clinical production capacity has benefited from development of highly productive cell lines and larger bioreactors using single-use technology, enabling the production of thousands of doses from a single batch of over 5 kilograms.

Today, we can accelerate these activities and enable production capacity for clinical studies for therapeutic mAbs. What could be the fastest path to provide mAbs for clinical evaluation during a pandemic outbreak? I propose that the answer could be 5–6 months, rather than 10–12 months.

Lead mAb identification and characteristics

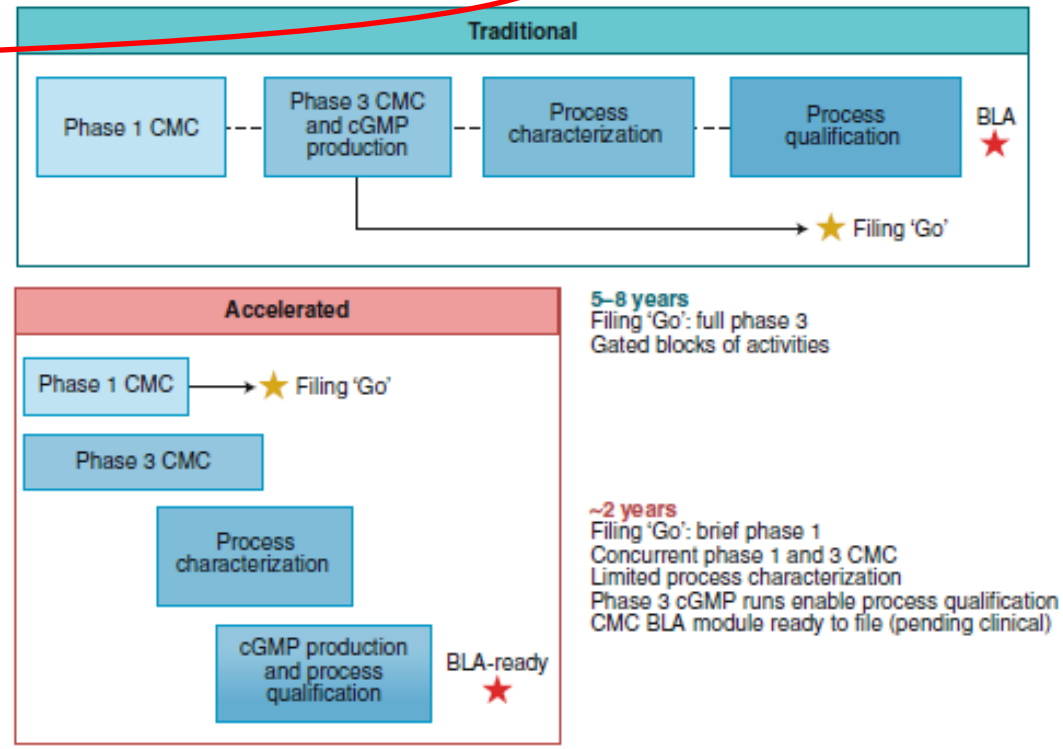
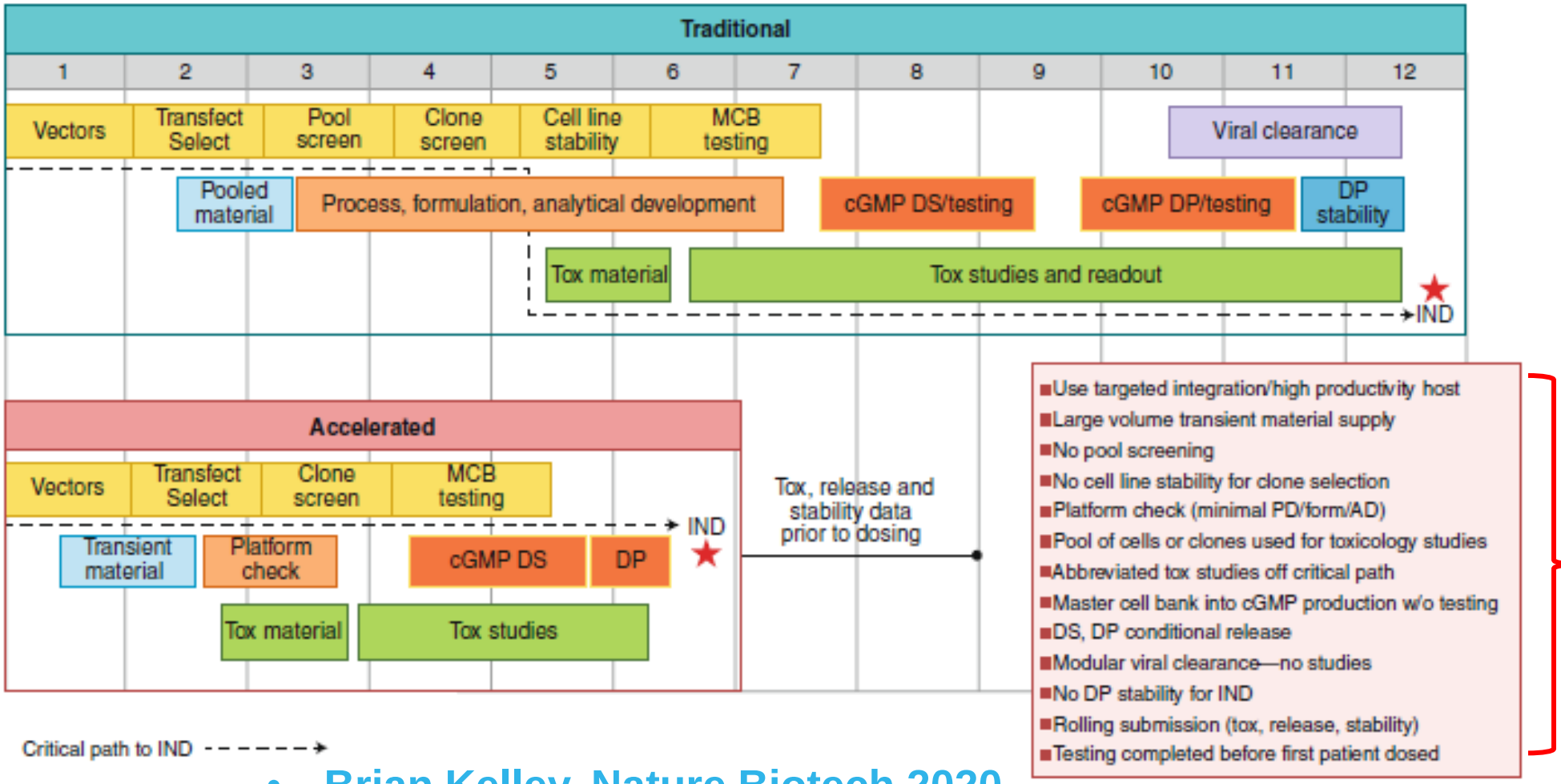


Fig. 2 | **mAb product development for pandemics (mAb lead identification to BLA).** The fastest path to licensure for therapeutic mAbs treating pandemic outbreaks requires accepting business risks and preinvestment, but without major product quality, supply and regulatory risks.

Accelerated PhI CMC timeline for a pandemic



Mass Spectrom.

- **Brian Kelley, Nature Biotech 2020**

Fig. 1 | **Accelerated phase 1 CMC mAb timeline for a pandemic.** The timeline to phase 1 clinical studies using mAb therapeutics for pandemic outbreaks can be substantially accelerated without heightened product safety risks as compared with current practice. Tox, toxicology; MCB, master cell bank; DS, drug substance; DP, drug product; PD, process development; form, formulation; AD, analytical development.

Proposed International Nonproprietary Name of Covid19 Drugs (WHO): special edition

WHO Drug Information, Vol. 34, No. 3, 2020

Proposed INN: List 124 – COVID-19 (special edition)

International Nonproprietary Names for Pharmaceutical Substances (INN)

Notice is hereby given that, in accordance with article 3 of the Procedure for the Selection of Recommended International Nonproprietary Names for Pharmaceutical Substances, the names given in the list on the following pages are under consideration by the World Health Organization as Proposed International Nonproprietary Names. The inclusion of a name in the lists of Proposed International Nonproprietary Names does not imply any recommendation of the use of the substance in medicine or pharmacy.

Lists of Proposed (1–117) and Recommended (1–78) International Nonproprietary Names can be found in *Cumulative List No. 17, 2017* (available in CD-ROM only). The statements indicating action and use are based largely on information supplied by the manufacturer. **This information is merely meant to provide an indication of the potential use of new substances at the time they are accorded Proposed International Nonproprietary Names.** WHO is not in a position either to uphold these statements or to comment on the efficacy of the action claimed. Because of their provisional nature, these descriptors will neither be revised **nor included in the Cumulative Lists of INNs.**

www.who.int

mAbs against SARS-CoV-2 : 4 Approved (+PhIII)

Table 1
Summary of monoclonal antibodies against SARS-CoV-2.

Clinical stage	Trial ID	Product name	Sponsor	Target
EUA	NCT04427501	LY3819253 (LY-CoV555)	AbCellera/Eli Lilly	SARS-CoV-2 S protein
EUA	NCT04425629 NCT04426695 NCT04452318	REGN-COV2 (REGN10933 + REGN10987)	Regeneron/NIAID	SARS-CoV-2 S protein
EUA	NCT04525079; NCT04593641; NCT04602000	CT-P59	Celltrion	SARS-CoV-2 S protein
EUA request submitted to FDA	NCT04545060; Activ-3 study	VIR-7831/GSK4182136	Vir Biotechnol./GlaxoSmithKline	SARS-CoV-2 S protein
Phase-3	NCT04507256 NCT04625725 NCT04625972	AZD7442	AstraZeneca	SARS-CoV-2 S protein
Phase-3	NCT04429529; NCT04649515	TY027	Tychan	SARS-CoV-2 S protein
Phase-3	NCT04479644; Activ-3 study	BRII-198	Brii Bio/TSB Therapeutics/Tsinghua University/the 3rd People's Hospital of Shenzhen	SARS-CoV-2 S protein
Phase-3	NCT04479631; Activ-3 study	BRII-196	Brii Bio/TSB Therapeutics/Tsinghua University/the 3rd People's Hospital of Shenzhen	SARS-CoV-2 S protein
Phase-3	NCT04483375; NCT04644185	SCTA01	Sinocelltech Ltd./Chinese Academy of Sciences	SARS-CoV-2 S protein

- **Barak PK, et al, Int J Biol Macromolecules 2021**
- **Reichert J, et al, mAbs 2022**

Covid-19 mAbs : accelerated R&D timelines

- The fastest timeline from lead mAb identification to First-In-Human Phase I studies is an important goal for all companies and the patients they treat.
- Many companies developing therapeutic mAbs have worked tirelessly to refine their technology and strategies to enable rapid clinical evaluation converging on a typical timeline for initial clinical studies.
- The combination of approaches based of a pandemic disease outbreak can reduce the time from the current standard of mAb lead identification from an already rapid IND of 10–12 months to potentially as little as 5–6 months as illustrated by successful Covid-19 mAbs (cf 4 FDA-EMA approved)
  - Kelley N, Nature Biotech 2020
  - Zheng Z et al Biotech Prog 2021
  - Agostinetto R et al, Biotech Bioeng 2021
  - Xu J et al, mAbs 2022
  - Reichert JR et al, mAbs 2022
- The combination of these acceleration strategies for addressing a pandemic outbreak could potentially be applied to clinical development for other mAbs & indications.

Cutting-edge analytical & structural network: antibody-based drugs (2005-22: +250 papers*, +280 talks)**

OptimAbs

2010-13

Université de Strasbourg

Laboratoire de Spectrométrie de Masse Bio-Organique LSMBO

LSMIS

Université de Genève

EPFL École polytechnique fédérale de Lausanne

Merck

Université de Lyon

isa Institut des Sciences Analytiques

Gustavus Gustavus Adolphus College

promise Advanced Proteomics

Spectroswiss

abbvie

Genovis

Quality Assistance Contract Research Organisation

SGS

Anaquant

Lonza Pharma & Biotech

ValenzaBio

FDA

Waters The science of what's possible.™

Bruker

Thermo Scientific

eurofins

Catalent

EspeRare

GTP Biologics CDMO

usp

Rapid Novor Speed up discovery

Peaks AB Antibody Characterization Service http://www.bioinfor.com/antibodyservice/

affilogic

IGENICA Biotherapeutics

World Health Organization

MAB Working Party

edqm European Directorate for the Quality of Medicines & HealthCare

ansm Agence nationale de sécurité du médicament et des produits de santé

NIST National Institute of Standards and Technology U.S. Department of Commerce

Chairman COSSF

MABDESIGN

Associate Editor

mAbs

* IF 60, +13,600 citations

** Open research & innovation

mAbs: 2009-2022 (Open access)

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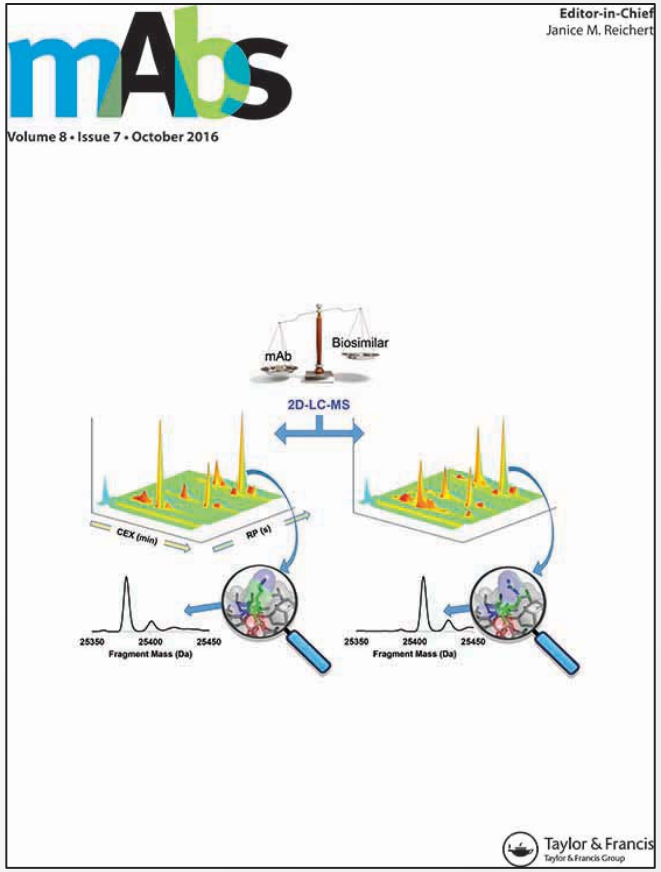

www.tandfonline.com/toc/kmab20/current

MABS
https://doi.org/10.1080/19420862.2018.1553476

(2019)

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REVIEW

Structure, het

Yingda Xu[a], Dongc
Wei Xu[g], Smita Ra
and Hongcheng Li

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Pharmaceuticals, Inc., N
Development, Regener
[g]Analytical Method Dev
USA; [i]Analytical Develo
[k]Product development,
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ABSTRACT
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MABS
2017, VOL. 9, NO. 8, 1217–1230
https://doi.org/10.1080/19420862.2017.1368602

(2017)

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REVIEW

Forced degrad

Christine Nowak[a], J
Gomathinayagam P

[a]Product Characterizatio
USA; [c]Biologics and Vacc
USA; [e]Millennium Resear
Chemistry, NBEs, Center

ABSTRACT
Forced degradatio
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MABS
2018, VOL. 0, NO. 0, 1–26
https://doi.org/10.1080/19420862.2018.1438797

(2018)

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REVIEW

Analytical comparability study of recombinant monoclonal antibody therapeutics

Alexandre Ambrogelly[a], Stephen Gozo[b], Amit Katiyar[c], Shara Dellatore[d], Yune Kune[e], Ram Bhat[f], J
Dongdong Wang[j], Christine Nowak[j], Alyssa Neill[j], Gomathinayagam Ponniah[j], Cory King[j], Bruce M
and Hongcheng Liu[j]

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Pharmaceuticals, 100 College Street, New Haven, CT ; [l]Analytical Chemistry, NBEs, Center d'Immunologie Pierre Fabre, St Ju
France

ABSTRACT
Process changes are inevitable in the life cycle of recombinant monoclonal antibody therapeutics. Products made using pre- and post-change processes are required to be comparable as demonstrated by comparability studies to qualify for continuous development and commercial supply. Establishment of comparability is a systematic process of gathering and evaluating data based on scientific understanding and clinical experience of the relationship between product quality attributes and their impact on safety and efficacy. This review summarizes the current understanding of various modifications of recombinant monoclonal antibodies. It further outlines the critical steps in designing and executing successful comparability studies to support process changes at different stages of a product's lifecycle.

White papers:
AbbVie
Adimab
Alexion
BioAnalytix
BMS
Gilead
Innovent
Merck
Millenium
Pierre Fabre
Regeneron

Dr. Hongcheng Liu

Pierre Fabre

Public

MABS
2022, VOL. 14, NO. 1, e2075078 (9 pages)
https://doi.org/10.1080/19420862.2022.2075078

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REPORT

OPEN ACCESS Check for updates

International nonproprietary names for monoclonal antibodies: an evolving nomenclature system

Sofia S. Guimaraes Koch[a], Robin Thorpe[b], Nana Kawasaki[c], Marie-Paule Lefranc[d], Sarel Malan[e], Andrew C.R. Martin[f], Gilles Mignot[g], Andreas Plückthun[h], Menico Rizzi[i], Stephanie Shubat[j], Karin Weisser[k], and Raffaella Balocco[a]

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ABSTRACT

Appropriate nomenclature for all pharmaceutical substances is important for clinical development, licensing, prescribing, pharmacovigilance, and identification of counterfeits. Nonproprietary names that are unique and globally recognized for all pharmaceutical substances are assigned by the International Nonproprietary Names (INN) Programme of the World Health Organization (WHO). In 1991, the INN Programme implemented the first nomenclature scheme for monoclonal antibodies. To accompany biotechnological development, this nomenclature scheme has evolved over the years; however, since the scheme was introduced, all pharmacological substances that contained an immunoglobulin variable domain were coined with the stem *-mab*. To date, there are 879 INN with the stem *-mab*. Owing to this high number of names ending in *-mab*, devising new and distinguishable INN has become a challenge. The WHO INN Expert Group therefore decided to revise the system to ease this situation. The revised system was approved and adopted by the WHO at the 73rd INN Consultation held in October 2021, and the radical decision was made to discontinue the use of the well-known stem *-mab* in naming new antibody-based drugs and going forward, to replace it with four new stems: *-tug*, *-bart*, *-mig*, and *-ment*.

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Member of EDQM MAB working group (2017-22)*

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2017, VOL. 0, NO. 0, 1–14
https://doi.org/10.1080/19420862.2017.1386824

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REPORT

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International standards for monoclonal antibodies to support pre- and post-marketing product consistency: Evaluation of a candidate international standard for the bioactivities of rituximab

Sandra Prior[a], Simon E. Hufton[a], Bernard Fox[a], Thomas Dougall[b], Peter Rigsby[b], Adrian Bristow[b], and participants of the study

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- αTNF
- Functional tests (Fab/Fc)
- SEC
- cIEF
- CE-SDS
- CZE

***EDQM (PhEur) +**

- **European National Competent Authorities (eg ANSM, PEI…)**
- **Australia, Canada, South Korea, Taiwan…**
- **AstraZeneca, Lilly, Lonza, Merck, Novartis, Pierre Fabre, Sanofi, UCB**

https://www.ema.europa.eu/en/partners-networks/eu-partners/eu-member-states/national-competent-authorities-human

Thank you for your attention





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- B. Akla, C. Beau-Larvor, N. Loukili et al
- N. Corvaïa, JF Haeuw, P. Lowe et al

CRDPF, Toulouse, FR

- C. Lasserre, N. Regent, MF. Laliberté et al
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Antibody Society/ mAbs, USA (12 papers)

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- S. Heinisch et al

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- D. Suckau, A. Resemann, W. Jabs et al

Waters, FR, UK, US (LC-MS prototypes) (2 papers)

- W. Chen, D. Lascoux, L. Denbigh, J. Gebler et al

+ many more (see publications)