Boosting mass spectrometry-based analytics for biopharma

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Curriculum Vitae

Christoph Johann Gstöttner was born on the 25th of January 1993 in Zwiesel, Germany. At the age of six he went to primary school Bodenmais, followed by the secondary school in Zwiesel where he graduated in 2011. Following this Christoph studied Biology in Regensburg, which he finished in 2014 with a bachelor work in the functional characterization of various RNA Polymerase I mutants. Hereafter he continued with a master in biology focused in the area of biochemistry. During his master he became more and more acquainted with proteins, either measuring there activity and stability, producing whole protein complexes recombinantly or analyzing these by mass spectrometry. The latter he performed during a internship which he conducted 2016 at Roche in Basel, where he also performed his practical work for his master thesis on the automation of protein analysis by using multidimensional LC hyphenated to mass spectrometry. He graduated in 2017 as Master of Science with an excellent grade. Because of his fascination for biopharmaceutical characterization by mass spectrometry he got enrolled in a Marie Curie Horizon 2020 PhD consortium, called Analytics for Biologics (A4B). He performed his PhD research at the Leiden University Medical Center in the Center for Proteomics and Metabolomics under the supervision of Prof. Dr. Manfred Wuhrer and Dr. Elena Domínguez Vega. During this time he developed in close collaboration with industrial partners methods for the structural characterization of antibodies and SARS-CoV-2 RBDs and developed an approach for the functional characterization of antibodies and Fc receptors. After his PhD he will continue this line as a postdoctoral researcher at the CPM in collaboration with Roche Diagnostics in Penzberg.

List of publications

1. **<u>Gstöttner C</u>**, Klemm D, Haberger M, Bathke A, Wegele H, Bell C, Kopf R. *Fast and Automated Characterization of Antibody Variants with 4D HPLC/MS*. Analytical Chemistry 2018, 90, 2119−2125 (**Chapter 2**).

2. Pot S*, **<u>Gstöttner C</u>***, Heinrich K, Hoelterhoff S, Grunert I, Leiss M, Bathke A, Domínguez-Vega A. *Fast analysis of formulated antibody-derived therapeutics by automated multidimensional liquid chromatography – mass spectrometry.* Analytica Chimica Acta 2021, 1184, 339015 (**Chapter 3**).

***equal contribution**

3. **<u>Gstöttner C</u>**, Reusch D, Haberger M, Dragan I, van Veelen P, Kilgour DPA, Tsybin YO, van der Burgt YEM, Wuhrer M, Nicolardi S. *Monitoring glycation levels of a bispecific monoclonal antibody at subunit level by ultrahigh-resolution MALDI FT-ICR mass spectrometry.* mAbs 2020, 12(1), 1682403 (**Chapter 4**).

4. Tyshchuk O*, **<u>Gstöttner C</u>***, Funk D, Nicolardi S, Frost S, Klostermann S, Becker T, Jolkver E, Schumacher F, Koller CF, Völger HR, Wuhrer M, Bulau P, Mølhøj M. *Characterization and prediction of positional 4-hydroxyproline and sulfotyrosine, two post-translational modifications that can occur at substantial levels in CHO cells-expressed biotherapeutics.* mAbs 2019, 11(7), 1219–1232 (**Chapter 5**).

***equal contribution**

5. **<u>Gstöttner C</u>**, Nicolardi S, Haberger M, Reusch D, Wuhrer M, Domínguez-Vega E. *Intact and subunit-specific analysis of bispecific antibodies by sheathless CE-MS.* Analytica Chimica Acta 2020, 1134, 18-27 (**Chapter 6**).

6. **<u>Gstöttner C</u>**, Vergoossen DLE, Wuhrer M, Huijbers MGM, Domínguez-Vega E. *Sheathless CE-MS as a tool for monitoring exchange efficiency and stability of bispecific antibodies.* Electrophoresis 2021, 42, 171–176 (**Chapter 7**).

7. **<u>Gstöttner C</u>**, Hook M, Christopeit T, Knaupp A, Schlothauer T, Reusch D, Haberger M, Wuhrer M, Domínguez-Vega E. *Affinity capillary electrophoresis – mass spectrometry as tool to un-ravel proteoform-specific antibody-receptor interactions.* 2021, manuscript under review (**Chapter 8**).

8. **<u>Gstöttner C</u>**, Zhang T, Resemann A, Ruben S, Pengelley S, Suckau D, Welsink T, Wuhrer M, Domínguez-Vega E. *Structural and Functional Characterization of SARS-CoV-2 RBD Domains Produced in Mammalian Cells.* Analytical Chemistry 2021, 93, 6839−6847 (**Chapter 9**)

9. Van Schaick G*, **Gstöttner C***, Büttner A, Reusch D, Wuhrer M, Domínguez-Vega E. *Anion exchange chromatography – Mass spectrometry for monitoring multiple quality attributes of erythropoietin biopharmaceuticals.* Analytica Chimica Acta 2021, 1143, 166-172.

***equal contribution**

10. **Gstöttner C**, Zhang T, Resemann A, Pengelley S, Suckau D, Asperger A, Wuhrer M, Domínguez-Vega E. *Recombinant SARS-CoV-2 Receptor Binding Domain: Comprehensive Top-Down Sequence Confirmation, Curation and O-Glycosylation Site Determination.* Bruker (Technical Note)

11. **Gstöttner C**, Zhang T, Resemann A, Pengelley S, Suckau D, Asperger A, Wuhrer M, Domínguez-Vega E. *Proteoform characterization of recombinant SARS-CoV-2 Receptor Binding Domains.* Bruker (Technical Note) (In preparation).

12. Bathke A, Bell C, **Gstöttner C**, Kopf R, Klemm D. *Rapid Online Characterization and Reduction of Protein Modifications Using Fully Automated Two-Dimensional High Performance Liquid Chromatography–Mass Spectrometry.* LCGC Europe 2018, Volume 31(1), 10–21.

13. Vergoossen DLE, Plomp JJ, **Gstöttner C**, Fillié-Grijpma YE, Augustinus R, Verpalen R, Wuhrer M, Parren PWHI, Dominguez-Vega E, van der Maarel SM, Verschuuren JJ, Huijbers MG. *Functional monovalency amplifies the pathogenicity of anti-MuSK IgG4 in myasthenia gravis.* PNAS 2021, 118(13), e2020635118.

14. Lock S, **Gstöttner C**, Wuhrer M, Dominguez-Vega E. *Monitoring exchange efficiency and stability of bispecific antibodies (BsAb) by Capillary Electrophoresis-Mass Spectrometry (CE-MS).* Sciex 2021, Document number: RUO-MKT-02-12642-A (Technical Note)

15. Komuczki D, Dutra G, **Gstöttner C**, Dominguez-Vega E, Jungbauer A, Satzer P. *Media on-demand: Continuous reconstitution of a chemically defined media directly from solids.* Biotechnology and Bioengineering 2021, 1-13.

16. Van Schaick G, Domínguez-Vega E, **Gstöttner C**, van den Berg-Verleg JH, Akeroyd M, Olsthoorn MMA, Wuhrer M, Heck AJR, Abello N, Franc V. N*ative structural and functional proteoform characterization of the prolyl-alanyl-specific endoprotease EndoPro from Aspergillus niger.* Journal of Proteome Research 2021, 20, 4875-4885.

17. Van de Bovenkamp FS, Dijkstra DJ, Borggreven NV, Pool J, Zuijderduijn R, Abendstein L, **Gstöttner C**, Nilsson SC, Holmdahl R, Gelderman KA, Blom AM, Domínguez-Vega E, Parren PWHI, Sharp TH, and Trouw LA. *Protecting cells from complement-mediated cytotoxicity using engineered bispecific antibodies.* (In preparation)

Bookchapters:

18. **Gstöttner C**, Kaur H, Wuhrer M. *Glycosylation analysis.* Chapter in: Monoclonal Antibodies Physicochemical Analysis. Elesevier 2021, 1st Edition.

19. **Gstöttner C**, Haselberg R, Wuhrer M, Somsen GW, Domínguez-Vega E. *Assessment of macro- and microheterogeneity of monoclonal antibodies using Capillary Electrophoresis hyphenated with Mass Spectrometry.* Chapter in: Capillary Electrophoresis-Mass Spectrometry: Methods and Protocols, Springer Methods in Molecular Biology in press.

PhD Portfolio

PhD student:	Christoph Johann Gstöttner
PhD period:	January 2018 – November 2021
Promotors:	Prof. Dr. Manfred Wuhrer
Co-Promotor:	Dr. Elena Domínguez Vega
Department:	Center for Proteomics and Metabolomics

Training Courses

Soft skills:

- A4B Summer School
- Personal effectiveness workshop and talent dynamics
- Leadership and Career Development
- Scientific Presentation and writing
- Legal requirements, industrial application and entrepreneurship
- Grant writing workshop
- Job application strategies

General training:

- BROK
- Basic Methods and Reasoning in Biostatistics
- EFTMS Course (Basic training in FT-ICR)
- Advanced FT-ICR User School
- Protein Chromatography – Engineering Fundamentals and Measurements for Process Development and Scale-up
- Top-Down and Middle-Down Characterization of Proteins

Awards and Grants:

- Innovation grant from Roche Diagnostics (Germany)
  Project title: “Automatized workflows for gene therapy product characterization”
  Co-applicant: 96.035 Euro
- Global Strategy Team (GST) grant from F. Hoffmann-La Roche (Switzerland)
  Project title: “Rapid antibody analysis using multidimensional separations and mass spectrometry”
  Co-applicant: 66.613 Euro
- CASSS Student Travel Grant
- Separations Travel Award
- Best Poster Award (EFTMS Conference)

Secondments:

- 2018 - Roche Diagnostics (Department Analytical Biochemistry Technical Development Europe & GLP Analytics (PTDEA), Penzberg, Germany)
- 2019 - Roche Diagnostics (Department Analytical Biochemistry Technical Development Europe & GLP Analytics (PTDEA), Penzberg, Germany)

Supervision

- Parnia Shams Ghahfarokhi (MSc)
- Sanne Pot (MSc)
- Sacha A. Boon (MSc)
- Annika van der Zon (MSc)

Scientific contributions in conferences:

Poster presentations:

- Coupling of capillary electrophoresis with FTICR-MS for enhanced characterization of intact proteins. **13th European Fourier Transform Mass Spectrometry (EFTMS)**, 2018, Freising, Germany.
- Sheathless CE-MS for the characterization of new therapeutic antibody formats. **Global CESI-MS Symposium**, 2018, Leiden, The Netherlands.
- How many chains can we see? A novel middle-down approach based on MALDI-FTICR MS for fast and easy characterization of modified bispecific antibodies **Chemistry as innovative Science (CHAINS), 2018** Chemistry, Veldhoven, The Netherlands.
- Characterization of monoclonal antibodies and their modifications by MALDI-(in-source decay)-FT-ICR MS. **1st European Top-Down Proteomics Symposium (TDP),** 2019, Paris, France.
- MALDI-(in-source decay)-FT-ICR MS for the fast characterization of new therapeutic antibodies. **Analytical Technologies Europe (ATEurope),** 2019, Dublin, Ireland.
- Studying the structure function relationship of new antibody-derived therapeutics using sheathless CE-MS. **Analytical Technologies Europe (ATEurope),** 2020.

Oral Presentations:

- Structural and functional characterization of monoclonal antibodies and new antibody formats by mass spectrometry. **Chemistry as innovative science (CHAINS)**, 2019, Veldhoven, The Netherlands.

- Affinity Sheathless CE-MS for the selective study of FcRn - Antibody interaction. **36th International symposium on microscale separations and bioanalysis (MSB)**, 2020.
- Structural and functional characterization of Spike-RBD produced in different organisms. **eNVMS meeting on Covid-19 Research**, 2021.
- Affinity sheathless-CE-MS as a new tool for the functional assessment of antibody - Fc receptor interactions. **40th International Symposium on the Separation of Proteins, Peptides & Polynucleotides (ISPPP)**, 2021.

Round tables:

- Phase-relevant Protein Characterization, **Analytical Technologies Europe (ATEurope)**, 2020 Virtual symposium, November 2020.

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Thank you, Simone, for welcoming and supervising me in the first period of my PhD. You taught me a lot about MALDI and especially FTICR. I learned (and still learn) plenty about this impressive and very complex instrument. It was nice to work with you on the first publications of my thesis. Thanks also to Yuri for reviewing my PhD thesis and give it the last shape.

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