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Boosting mass spectrometry-based analytics for biopharma Gstöttner, C.J.

Citation

Gstöttner, C. J. (2021, November 30). *Boosting mass spectrometry-based analytics for biopharma*. Retrieved from <https://hdl.handle.net/1887/3245884>

Version: Publisher's Version

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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 their activity and stability, producing whole protein complexes recombinantly or analyzing these by mass spectrometry. The latter he performed during an 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. **Gstöttner C**, Klemm D, Habegger 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*, **Gstöttner C***, 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. **Gstöttner C**, Reusch D, Habegger 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*, **Gstöttner C***, 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. **Gstöttner C**, Nicolardi S, Habegger 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. **Gstöttner C**, 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. **Gstöttner C**, Hook M, Christopeit T, Knaupp A, Schlothauer T, Reusch D, Habegger M, Wuhrer M, Domínguez-Vega E. *Affinity capillary electrophoresis – mass spectrometry as tool to unravel proteoform-specific antibody-receptor interactions*. 2021, manuscript under review (**Chapter 8**).
8. **Gstöttner C**, 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. *Native 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*. Elsevier 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.

Acknowledgments

First of all, I would like to thank everyone who is able to attend my defence during this difficult corona situation. But also, I want to thank you all for the last 4 years where I learned a lot and had a lot of fun.

A great thanks goes to my supervisor Manfred, who enabled me to perform a PhD in his laboratory. You always supported me during our meetings, especially those with Roche where we all could brainstorm and come up with new ideas and projects. Thanks for critically questioning my ideas, but also for giving me the support necessary to realize them. Thanks also for giving me feedback and thereby allow me to strengthen my weaknesses.

A special thanks also to my co-promotor Elena who always supported me during the 4 years. You always trusted me in the lab and really gave me freedom to develop my own ideas, especially with the mD-LC. I learned a lot from you about MS but muuuuuch more about CE. I remember still the moment when we tried for the first time affinity CE-MS. I was really amazed and surprised that it worked out so nice. You were a great supervisor, not only in respect to my PhD, but even more importantly you prepared me perfectly for my future career.

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.

I would like to thank my amazing paranymphs, Guusje and Steffen. Especially Guusje, not only for guiding me through my defence but also because you taught me a lot about Dutch culture and beautiful places (Achterhoek, Zeeland (Kokkel and Oyster collecting), Amsterdam...). You were a great officemate and friend and I really enjoyed the time we had together. Also, I would like to thank my unofficial paranymphs Willem and Elena, which supported Guusje making possible that I defend together with Steffen.

A special thanks also to Wei and Di, who explained me so much about China. I enjoyed cooking together Hotpot or going to the beach for a swim with the inflatable crocodile. Here again a great thanks to the local of Katwijk, Willem, which knows the best hidden beach spots, where 18 degrees feel like a hot summer day. Also, you were always a good friend and we had several 'hikes' in the dunes together, which I enjoyed a lot!

Thanks also to all the students (and nearly PhD student) which I supervised in the last 4 years, Annika and Sacha, Parnia and Sanne. It was very nice working with all of you. I also learned guiding people and planning projects. I enjoyed working with all of you and wish you good luck on your future way.

Thanks to my consortium and to all my A4B colleagues for the nice training events and meetings we had in the course of the almost 4 years.

Ein herzliches Dankeschön an German #2, Büronachbar, A4B Partner, Paranymphe und Freund, Steffen. Unsere Geschichte reicht schon weit zurück, als wir beide noch Roche Praktikanten waren und zudem im selben Haus lebten. In Basel aber auch hier in den Niederlanden hatten wir eine super Zeit. Wir sind viel gereist und übernachteten in den verrücktesten Unterkünften, entweder mit dem Gefühl, dass sich der Flughafen oder Bahnhof in unserem Hotelzimmer befindet, auf einem Boot mit Sauna oder einem Schloss. Alles Gute in SSF.

Ein riesiges Dankeschön für die super Zusammenarbeit an die Kollegen von Roche. Vor allem Dietmar und Markus Habegger vielen Dank für die Hilfe in diversen Projekten. Es war super schön, wenn ihr in Leiden wart, bzw. wir in Penzberg. Vielen Dank auch an Michaela für die unzähligen Proben die du nach Leiden geschickt hast. Des Weiteren Dankeschön an Robert, Anja, Ingrid, Sina, Katrin, Raphael, Michi und Markus Haindl für die gute Zusammenarbeit. Vor allem vielen Dank an Dietmar, Michi und Christian, für eure sehr hilfreichen Ratschläge vor aber auch während meinem PhD!

Ein herzliches Dankeschön auch an meine Familie und Freunde, die mich immer unterstützt haben und mich zu Hause immer wieder zur ein oder anderen Wanderung herzlich empfangen haben. Vielen Dank Papa, Mama und Reinhold, dass ihr mir auf meinem Weg immer geholfen habt, egal wohin die Reise ging, entweder nach Regensburg, Basel, Penzberg, Stuttgart oder nach Leiden. Die letzte Zeit war ein bisschen schwierig, da wir uns wegen Corona nicht bzw. nur online sehen konnten, aber es kommen sicher wieder andere Zeiten und mal sehen, wo die Reise als nächstes hingeht.