Institute of Bioprocess Science and Engineering

Annual Report 2022

March 2023

Table of Content

Introduction

The year 2022 was very productive for our Institute although universities in general were confronted with a financially challenging environment. A major contribution to our success was third-party financing raised from both public funding institutions and corporate partners. All ongoing research projects were successfully executed, which is reflected in the high number of scientific publications, conference contributions, and completed bachelor's, master's, and doctoral theses supervised at our institute in 2022.

We are very optimistic for the future development of IBSE. Linking the university funding to corresponding performance indicators will guarantee adequate financial support for our institute from BOKU side in the next years, regardless of tight university budgets. We see a steadily growing demand for graduates with sound scientific expertise in bioprocess engineering in industry and academia. IBSE's approach of working on bioprocess engineering issues on a fundamental scientific basis and translating knowledge into new process approaches and innovations is ideally suited for developing scientifically critical, highly qualified scientists who are very well prepared for the job market.

IBSE was very successful in 2022 and we significantly strengthened our positioning in the field of basic research in bioprocess engineering through 3 successfully acquired FWF projects and a new CD lab.

In May 2022 the DocSchool Bioprocess Engineering was evaluated by a team of international reviewers. As IBSE faculty is very prominently represented in the faculty of the DocSchool we were happy to receive a very positive evaluation report from the reviewers and the BOKU financing of the DocSchool was prolonged for another five years. We thank the former head of the DocSchool Alois Jungbauer for all his effort and the DocService for their ongoing support!

In 2022, IBSE has experienced major personnel changes with the promotion of Gerald Striedner to a §99.4 professor in bioprocess engineering and the appointment of Johannes Buyel as the new §98 professor in the field of downstream processing. We are also pleased to announce the successful habilitation of Peter Satzer in the field of bioprocess engineering. In general, major changes of personnel are often associated with challenges at the scientific and organizational level of an institution. After the first months together with Johannes, the clear conclusion is that IBSE has gained a great colleague; very appreciative in his personal dealings and extremely well qualified in scientific matters. Johannes complements the existing expertise in bioprocess engineering at IBSE in the best possible way. He brings new scientific aspects to IBSE with his expertise in process modeling, data science and plant biotechnology. The smooth transition in the bridging phase was also strongly supported by Professor Alois Jungbauers willingness to still contribute with his expertise and network.

Finally, we would like to take the opportunity to thank all our project partners, the IBSE staff and the students working with us for their commitment, and willingness to contribute, share and collaborate. With this in mind, we look to the future with optimism, knowing that a lot of work and many challenges lie ahead. Our scientific curiosity coupled with our high motivation and excellent team spirit will enable us to master these challenges with much joy and success.

Gerald Striedner

Astrid Dürauer

Introduction of Johannes Buyel

Dear cooperation partners, colleagues and friends, about a year ago, in January 2022, I first set foot into BOKU as part of the negotiations to become the new professor for downstream processing at IBSE, not knowing how things would go. It was quite clear that joining IBSE meant big footsteps to fill, but from the start I really enjoyed the open-minded and heartly welcome at the institute. From my point of view, this welcoming atmosphere has since grown into mutual trust and I am very thankful to all of you for integrating me into so many of the daily routines and new projects alike.

In terms of the latter, I would like to use this opportunity to briefly outline my agenda, interests and project ideas for the next years. To do this, I will first start with a short presentation of my background before digging deeper into details.

I am a biotechnologist and bioprocess engineer by training with a focus on plant molecular farming (i.e. the production of recombinant proteins and small molecules in plants and plant cells), downstream processing and process modelling. My interests also include data extraction, processing and handling as well as statistical experimentation, i.e. design of experiments (DoE). Furthermore, I very much enjoy teaching and helping young scientist to develop their research skills ultimately becoming independent investigators.

In this context, I would like to complement the upstream capabilities at IBSE and the DBT by bringing plant-based expression to the table. For example, with Janos Bindics as a driver, we have started an acib-funded project to assess the potential of poppy plant cell culture to produce opioids. We plan to augment these activities through cooperation with both company partners, e.g. the Medicines-4-future (M4F) initiative, and colleagues from academia, e.g. the TU and MedUni Graz. These projects will be centered around recombinant protein production, including purification, for example of growth factors for advanced therapy medicinal products (ATMP) and cultured meat production but also bionanoparticles and toxins for cancer therapy.

In the downstream context, I plan to build a working group focusing on mechanistic and hybrid chromatography modeling. This will include the implementation of CADET as a tool to predict protein separation using mass transfer and isotherm models for which we will develop novel isotherms and methods for quick parameter determination. Another aspect is capturing relevant protein (surface) properties using simple mechanistic descriptors for which we will screen various approaches like the Laplace-Beltrami operator. An according grant proposal is in preparation. Furthermore, as part of recently initiated projects, I intend to attract additional researchers to characterize process variability, to develop quick responses against biological threats and to investigate the purification of amino acids from crude feeds.

On the teaching side, I will continue to overhaul existing lectures to incorporate additional interactive elements with the goal to foster transfer and context knowledge and balance methodological skills with expert knowledge. A major topic in this context will be the impact of digitalization both on teaching and learning methods but also on the necessary expert skills, e.g. modeling capabilities.

In terms of administration and organization, I plan to support the department in introducing a digital laboratory notebook. If possible, with an option to implement data analysis routines and data banking. As if the list of to-dos was not long enough already, my mid-term goal is to initiate a long-term partnership with suitable schools to facilitate a seamless transition and accelerated start for pupils joining BOKU.

I know that these are ambitious plans and failure in some is an acceptable option. However, I am sure that with your support and joined efforts a substantial and relevant part of these ideas can be set into motion and turned into application. I will do my best to communicate my thoughts as clearly as possible and I cordially invite you to share your concerns, ideas and thoughts about them with me!

Introduction of Peter Satzer

End of 2022 I habilitated in the field of Bioprocess Engineering at the BOKU with a broad expertise in bioprocess engineering in Upstream and Downstream with a focus on continuous production and biopharmaceuticals. Especially in the last years of my scientific development, the focus shifted to future opportunities and challenges in bioprocess engineering: Sustainability and life cycle analysis of bioprocesses, 3D printing for biotechnology, and novel drug-formats for gene therapy and vaccine production.

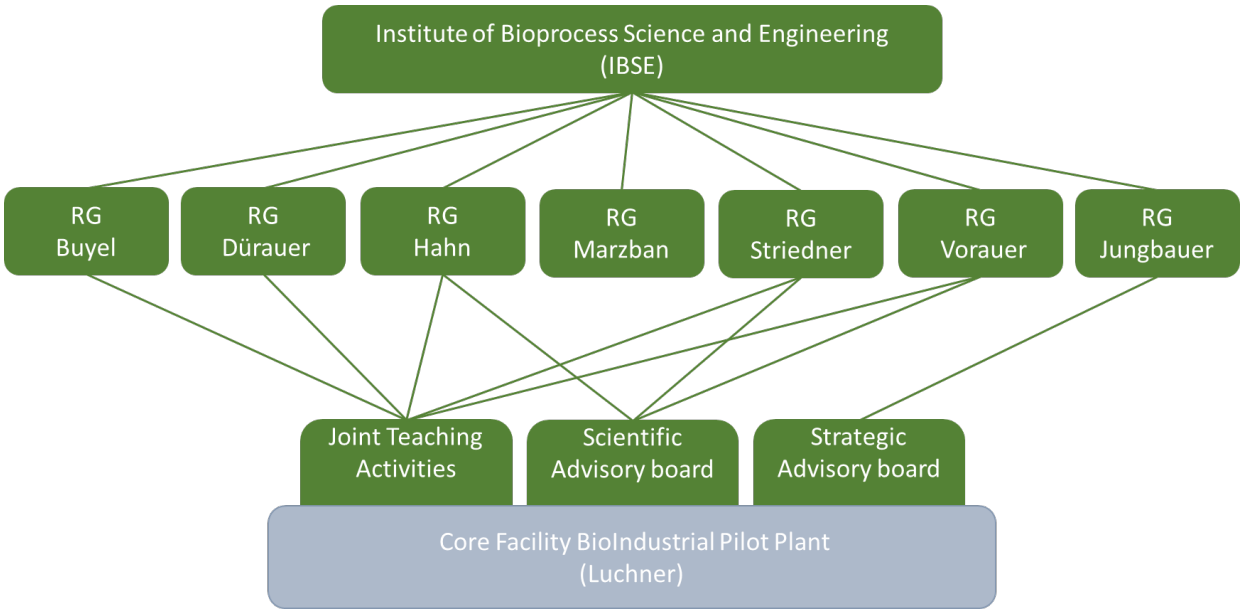
I am grateful to take the opportunity at IBSE to pursue my future scientific development as an independent research group, and in light of the future challenges and the main focus of BOKU as a whole, my future work will be directed towards sustainable bioprocess development. In the past I have already worked on the life cycle assessment of bioprocesses, defining new key-characteristics for describing the sustainability of bioprocesses, and implementing biodegradable plastics into everyday work in life-science laboratories. I think driving biotechnological research to contribute to the climate goals for a sustainable future is a key mission for BOKU and IBSE in the future.

To enable this development for IBSE and my personal scientific development, a number of project proposals have already been submitted and are waiting for assessment, including a FWF-START Proposal to establish a new LCA framework for biotechnology and an opn2EXPERTS call by Boehringer Ingelheim on the subject of making buffer selection in chromatography more sustainable. To explore the opportunities of 3D printing in biotechnology on a scientific and education level, a MSCA-DN was submitted with myself as a coordinator on the topic of 3D printing in biotechnology, and an FWF-Einzelproject was submitted on the topic of 3D printed surfaces and their influence on cell attachment. A number of other project opportunities are currently discussed with various company partners, and inclusion of LCA of bioprocesses will be a future part of other projects in IBSE.

To contribute to sustainability on a more organizational level, I'm currently in the process of integrating IBSE into the BOKU LCA-Platform to enhance internal collaboration as there is much to learn for bioprocess LCA from fields like agricultural, environment and food technologies which are more developed in that regards. Additionally, I have taken up the position of Environmental, Health and Safety Speaker for the DBT and will work to implement measures to reduce plastic waste, water usage and energy consumption in the labs by first implementing and testing them on working group/institute level and then scaling to the department or wider BOKU context. For instance, currently we are working on integrating into the Green Labs Austria initiative for plastic waste recycling in life science laboratories.

On the teaching side, I will contribute to the topics of continuous production in dedicated lectures, and will supplement the technical orientation of lectures that I participate in, for instance the biotechnological seminar, with aspects of sustainability, equality and social impact of discussed technologies.

Structure of the *Institute of Bioprocess Science and Engineering* 2022



Members of the Research Groups 2022

RG Buyel			
Staff	**PhD student**	**BA/MA student, intern**	**Technician**
Janos Bindics Johannes Buyel			
RG Dürauer			
Staff	**PhD student**	**BA/MA student, intern**	**Technician**
Astrid Dürauer	Ignacio Montes Serrano Bettina Motycka Valentina Ruocco	Tobias Kargl Dominik Kallinger (BA) Katharina Somogyi	Eva Berger Irfan Erdem
RG Hahn			
Staff	**PhD student**	**BA/MA student, intern**	**Technician/Student assistant**
Rainer Hahn Nico Lingg Daniel Komuczki	Jürgen Beck Markus Berg Anna Frank Nils Gehrmann Alexander Jurjevec Matthias Müller	Alexander Mechtler (MA) Georg Hochdaninger (MA) David Scheich (MA)	Kerstin Holzer Timo Kalchmayr Vanessa Przybylowicz
RG Jungbauer			
Staff	**PhD-student**	**BA/MA student, intern**	**Technician**
Alois Jungbauer Patricia Pereira Aguilar	Lena Achleitner Johanna Bacher Mafalda Dos Santos Gregory Dutra Touraj Eslami Anna-Carina Frank Leo Jakob Daniel Komuczki Narges Lali Viktoria Mayer Magdalena Pappenreiter Carme Pons Royo Gabriele Recanati Alexander Zollner		Andreas Fischer Osama Mesef Magdalena Mosor Patrick Scheidl Willibald Steinfellner

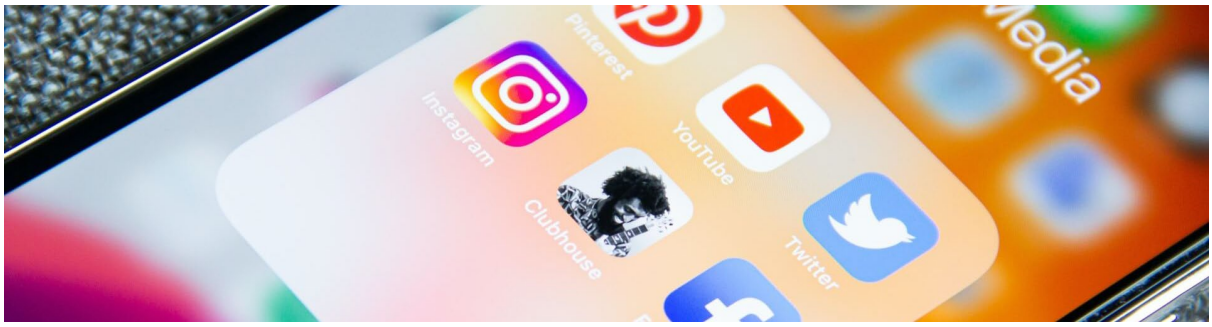
RG Marzban			
Staff	**PhD student**	**BA/MA student, intern**	**Technician**
Gorji Marzban	Sonja Schürer-Waldheim		

RG Striedner			
Staff	**PhD student**	**BA/MA student, intern**	**Technician/ Student assistant**
Monika Cserjan Mathias Fink Peter Satzer Gerald Striedner Christopher Tauer Birgit Wiltschi	Natalia Danielewicz Tommaso de Santis Lisa Fohler Martin Gibisch Stefan Gutmann Hana Hanaee-Ahvaz Arasteh Kani Arshia Marco Klanschnig Christoph Köppl Claudia Lacombe Zana Marin Florian Mayer Florian Strobl Sophie Vazulka	Martina Baumann (MA) Johanna Berein (MA) Felix Faschingeder (MA) Hugo Franca (MA) Pawel Gorecki (MA) Benedikt Haslinger (MA) Lea Jusufagic (MA) Lukas Leibetseder (MA) Roman Liebhart (MA) Karoline Reznar (BA) Anton Shpylovyi (MA) Florian Simon (MA) Lina Vranizky (MA) Martina Winter (MA)	Alexander Doleschal Moritz Dielacher Miriam Knees Lukas Leibetseder Helene Brenner Rebecca-Oana Pitik Lisa Letschnig Karoline Reznar Anton Shpylovyi Roman Liebhart Benedikt Haslinger

RG Vorauer-Uhl			
Staff	**PhD student**	**BA/MA student, intern**	**Technician**
Karola Vorauer-Uhl	Sarah Übleis (Co-supervisor) Ehsan Suleiman Yuelang Yao	Konstanze Kastenhofer Magdalena Kößlbacher Jasmina Memic Simon Nendwich	Gabriele Lhota

Core Facility: BioIndustrial Pilot Plant			
Staff	**PhD student**	**BA/MA student, intern**	**Technician/Student assistant**
Markus Luchner Mathias Fink		Alina Destinger (MA) Lorenz Haider Magdalena Hohlrieder Marco Kaupe Alexander Mechtler	Sabine Necina Philipp Peter Anita Zwanzleitner

IBSE goes Social Media



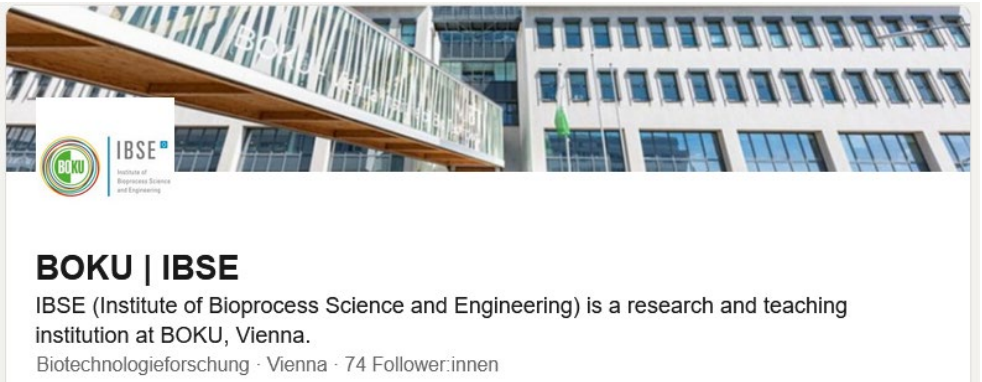
In 2022 our Institute finally has arrived in the 21st century by setting up three social media accounts: Twitter, LinkedIn and Instagram. These accounts are accessible through the same name:

@BOKU_IBSE

Our different channels aim to represent different academic aspect of our Institute: On **Twitter** we are posting our Institute's latest publications (eg. papers etc.) to gain visibility in the broader scientific field. On **LinkedIn** we are sharing guest talks, workshops, experiences from conferences, announcements and special celebrations such as awards and special lectures. This channel is our professional public image of our Institute, besides our BOKU Homepage. We are aiming to expand our professional network and gain more visibility in the biotech field.

Lastly, on **Instagram**, we are sharing the social side of our Institute by posting everyday happenings such as experiments, maintenance work, social happenings (like our famous cake challenges, get-togethers etc.). With this we want to reach out to future students and also play around with the not so serious side of academic research life.

Let's connect through one of these channels! Like, share and subscribe!



Success Stories

Project: Chromatographic fundamentals

Journal of Chromatography A 1673 (2022) 463058

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Alkaline treatment enhances mass transfer in Protein A affinity chromatography

Nico Lingg[a], Andreas Daxbacher[a], Desiree Womser-Matlschweiger[a], Dietmar Pum[b], Jürgen Beck[a], Rainer Hahn[a,*]

[a] Department of Biotechnology, Institute of Bioprocess Science and Engineering, University of Natural Resources and Life Sciences, Vienna, Muthgasse 18, Vienna 1190, Austria
[b] Department of Nanobiotechnology, Institute of Biophysics, University of Natural Resources and Life Sciences, Vienna, Muthgasse 11, Vienna 1190, Austria

Previously, non-intuitive changes in dynamic binding capacity after alkaline treatment have been observed for novel Protein A resins, where sharper breakthrough curves and increased capacities were reported. In this work, we have systematically investigated resins with both low and high alkaline stability and studied the changes in static and dynamic binding capacities and elution behavior. An example is shown in Figure 1. While the DBC10% of MabSelect Sure decreased to less than half of its initial value, both MabSelect PrismA and Toyopearl Protein A 650F show a slight increase in DBC.

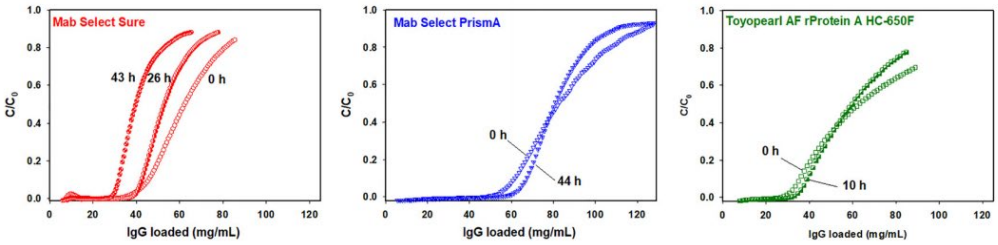


Fig. 1: BTCs with IgG on MabSelect SuRe (left panel), MabSelect PrismA (middle panel), Toyopearl AF-rProtein A HC-650F (right panel) after varying incubation time with 1 M NaOH. Column height was ~10 cm, velocity was 100 cm/h.

To obtain further insight into changes of intraparticle transport, intraparticle antibody uptake curves were measured by Confocal Laser Scanning Microscopy (Figure 2).

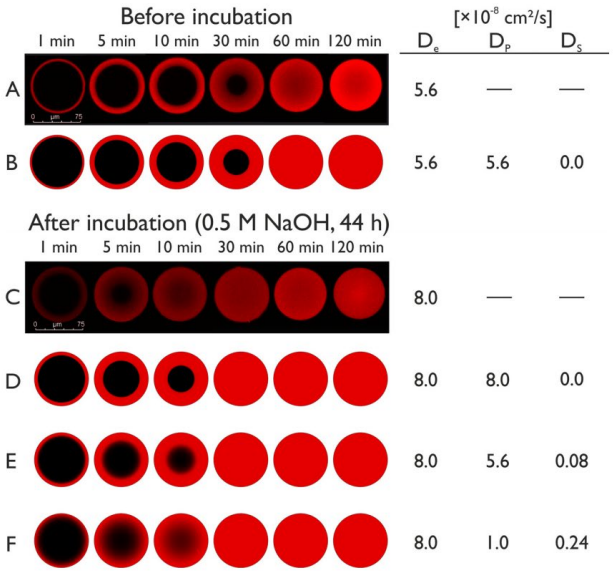


Fig. 2: CLSM of MabSelect PrismA before and after alkaline treatment. A and C show experimental CLSM pictures, whereas B, D, E and F represent artificial CLSM images. The observed effective pore diffusion coefficient De from BTCs is given for the experimental CLSM pictures. For the artificial images, the pore diffusion coefficient Dp and the solid diffusion coefficient Ds of the underlying model are given.

Under normal conditions MabSelect PrismA exhibits sharp profiles with a shrinking core which is typical for pore diffusion and highly favorable isotherm. Upon alkaline treatment, the profiles do not show sharp fronts but progressively become more diffuse (smoother) and eventually reach the core of the particles much faster. Artificial CLSM images were used to uncover the underlying transport mechanisms observed in Figure 2. Pore and solid diffusion parameters were selected to match the overall observed D_e values from BTC experiments and to match the experimental CLSM images. Based on the obtained values we propose that the observed mass transfer increases of up to 40% are due to a switch in diffusion mechanism. Based on our results, only a small window of alkaline treatment conditions exists, where dynamic binding capacity can be increased. Our findings may help to explain previous findings and observations of others.

Project: acib94041: From Micro- to industrial Scale; Scaling of downstream processing–how small is big enough?

Markus Berg, Eva Berger, Irfan Erdem, Rainer Hahn and Astrid Dürauer

The goal of this project is to develop predictive models for scale up/down using recombinant hFGF-2 in E. coli as model system. For this purpose, detailed characterization of unit operations with advanced analytics were carried out. The scale ranged from micro-fluidic stations up to pilot scale processing. We have studied cell harvest, cell disruption, clarification and chromatographic capture with instruments typical for the respective scale. Special focus was set on the intercorrelations between the different unit operations as outlined in the Figure below.

A main focus is the study of clarification by filtration at which load and filtrates were characterized by different particle size distribution methods. A main result of these investigations reveal that host DNA plays a major role in membrane and filter blocking when processing E. coli homogenates.

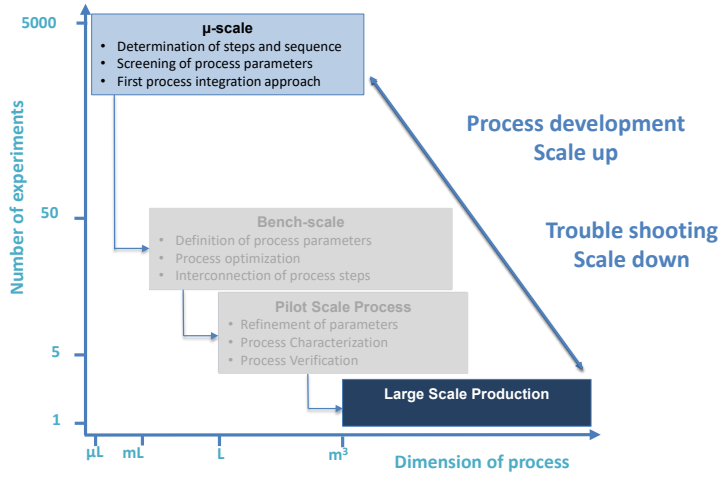


Fig. 1: Representative scheme for scaling from micro to industrial scale and vice versa.

We characterized our capture step of hFGF-2 on Capto S cation exchanger by extensive chromatographic experiments and modelling. Our studies revealed that mass transfer in crude solutions, e.g. homogenates, is about a factor of 40 slower compared to the pure protein solution. Results were published in Journal of Chromatography.


Journal of Chromatography A 1676 (2022) 463264

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Mass transfer of proteins in chromatographic media: Comparison of pure and crude feed solutions

Markus C. Berg[a], Jürgen Beck[b], Alex Karner[b], Kerstin Holzer[b], Astrid Dürauer[a,b], Rainer Hahn[a,b,*]

[a] Austrian Center of Industrial Biotechnology, Muthgasse 18, Vienna 1190, Austria
[b] Department of Biotechnology, Institute of Bioprocess Science and Engineering, University of Natural Resources and Life Sciences Vienna, Muthgasse 18, Vienna 1190, Austria

Project: A fully automated, integrated and continuous End-to-End platform process skid for biomanufacturing (End2End)

Alois Jungbauer, Gabriele Recananti, Magdalena Pappenreiter, Gerald Striedner and Bernhard Sissolak

Continuous manufacturing of biopharmaceuticals has become a very topical issue as it offers numerous opportunities to improve process economics and reduce the environmental footprint. The supply chain disruption during the pandemic demonstrated that efficient and simple manufacturing systems are the best solution to maintain production during difficult times. For more than a decade, several continuous antibody manufacturing projects were carried out at IBSE and the foundation was laid for an FFG-funded frontrunner project with the company Bilfinger. The main objectives of the project were to design, construct and test a holistic "integrated" end-to-end platform process (for biotechnological plants with continuous material flow for the production of high-value biopharmaceuticals) for a campaign of more than 20 days.

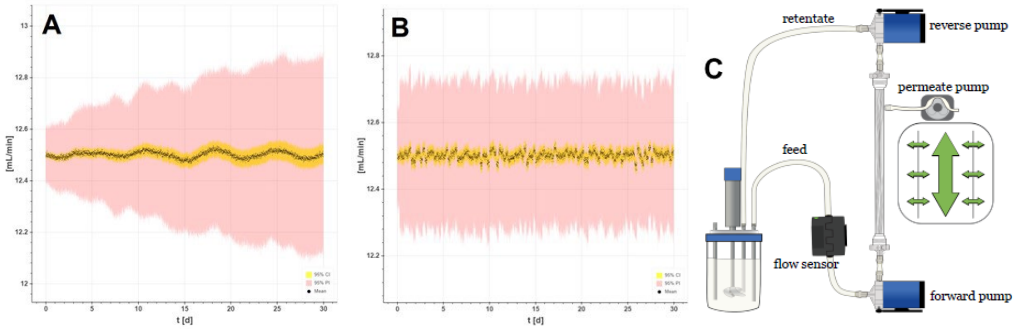


Figure 1 Example of model predictive control of harvest flow rate of perfusion reactor with (A) conventional model feedback control and (B)model predictive control where the harvest flow is maintained constant over the entire manufacturing campaign; adapted from Pappenreiter, M., Döbele, S., Striedner, G., Jungbauer, A., Sissolak, B. Model predictive control for steady-state performance in integrated continuous bioprocesses (2022) Bioprocess and Biosystems Engineering, 45 (9), pp. 1499-1513; () schematic depiction of a perfusion system which is integrated in our end2end system, adapted from Pappenreiter, M. Schwarz, H. Sissolak, B. Jungbauer, A. Chotteau, V. Product Sieving of mAb and its High Molecular Weight Species in different modes of ATF and TFF Perfusion Cell Cultures, (2023) Biochemical Engineering Journal, published on line

To develop an innovative automated precipitation method for the capture step, an Advanced Process Control (APC) regime, to fully automate the operation of the system to provide a robust process. The process consists of a perfusion culture directly connected to a capture unit, followed by virus inactivation and polishing. In the perfusion culture, flow rates and/or product concentration fluctuate. These process disturbances carry over to the entire process. To stabilize the product, a model predictive control system was developed to ensure stable production of the biopharmaceutical (Figure 1). After testing the integrated process in the laboratory with continuous production of antibodies, we started to develop a process skid as a prototype for industrial production of biopharmaceuticals in a continuous manner. We called this form dream to reality (Figure 2) because our first publication on continuous biomanufacturing originate from the years 2005. We will generate a show case for full automation and control with integration of advanced on-line monitoring using conventional and advanced sensors.

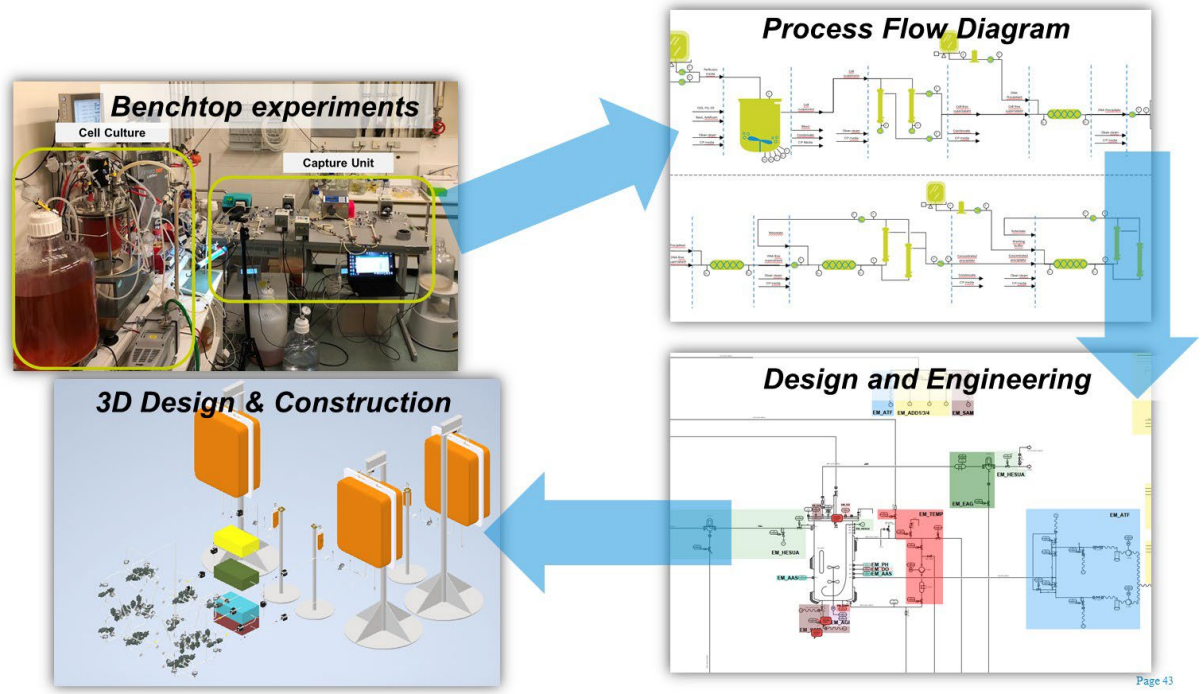


Figure 2: Prototype construction for continuous membrane-filtration based continuous processing in an end2end production system processing; Prototype testing at benchtop scale (2L perfusion reactor); Upscale to 10L perfusion process

In frame of two Marie Curie innovative training networks (A4B and Codobio) we have expanded the ideas of continuous biomanufacturing and developed a system where the precipitant is added as a solid. The idea was tested at small scale laboratory and then an in-depth economical and

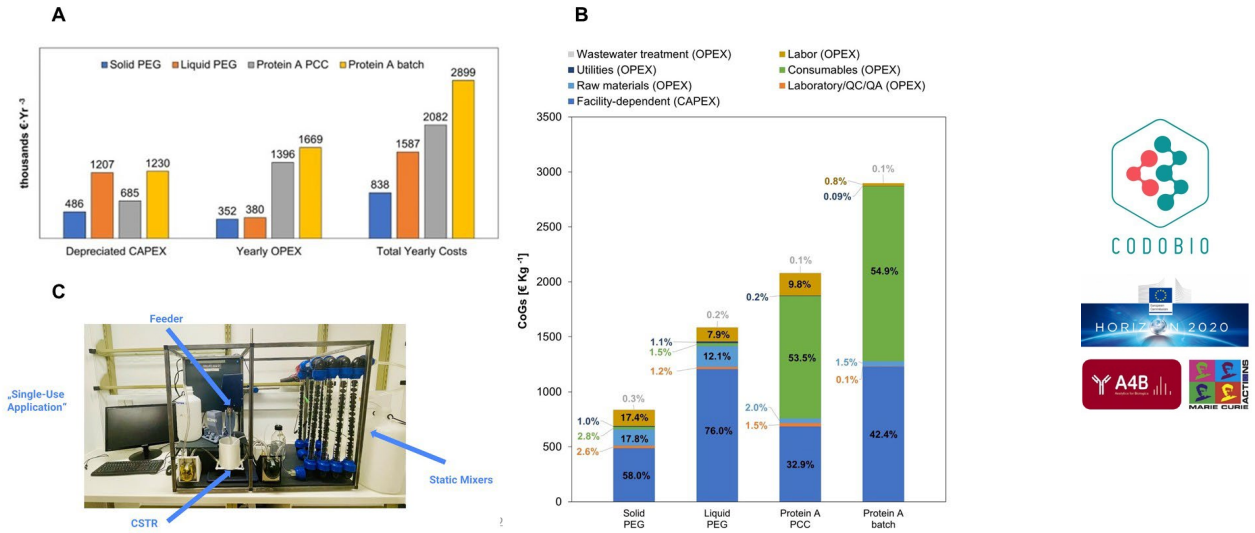


Figure 3: Cost calculation for a continuous antibody purification (A) split in capital expenses (CAPEX), operating expenses (OPEX) and total costs, (B) composition of to the cost of goods (CoGs) and (C) prototype eipiment for the continuous addition of precipitant. Data from Pons Royo, M.D.C., De Santis, T., Komuczki, D., Satzer, P., Jungbauer, A. Continuous precipitation of antibodies by feeding of solid polyethylene glycol (2023) Separation and Purification Technology, 304, art. no. 122373,

environmental analysis was performed (Figure 3) which showed that it is possible to reduce the cost of goods below € 1000 per gram recombinant antibody. If we are able to recycle the precipitant then we are in the range of € 300 per gram which is then an affordable process for low- and middle-income companies and application of antibodies for anti-venoms and passive immunization.

Project: CD Laboratory for production of next-level bio in *E. coli*

CRISPRactivation-SMS, a message for PAM sequence independent gene up-regulation in *Escherichia coli*

The success of CRISPRa-mediated gene up-regulation in the bacterial genome is highly limited by the availability of a PAM sequence at specific genomic locations. However, most endogenous genes do not have a PAM sequence exactly at these ideal sites. We could overcome these limitations by combining the recently engineered PAMless Cas9 variant **S**pRY (Walton, R.T. *et al*. 2020), with a CRISPRa construct using phage protein **M**CP fused to transcriptional activator **S**oxS (Dong, C. *et al.* 2018). The CRISPRa construct, named **SMS**, was tested on various levels for its PAM independency.

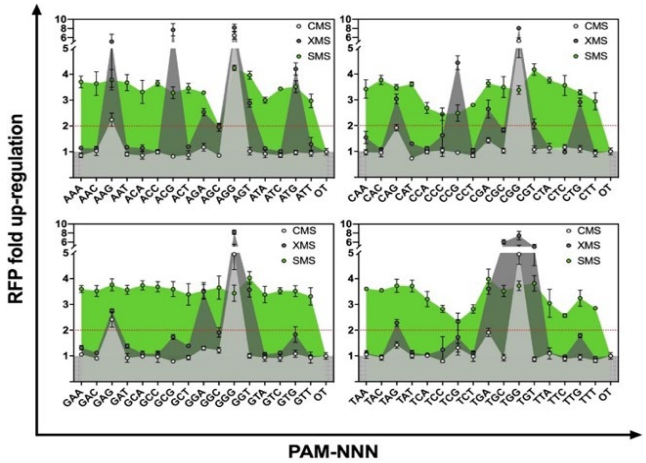


Figure 4: PAM determination assay confirmed PAM independency for SMS

We tested the capacity of three MCP-SoxS based CRISPRa constructs using either dCas9 (CMS), dxCas9 (XMS), or dSpRY (SMS) for the up-regulation of a weak expressed red fluorescent protein (RFP). For this purpose, we built a PAM library, containing all 64 possible PAM sequences on a plasmid, located at position -81 bp upstream the transcriptional start site (TSS). By testing the up-regulation level of RFP, we demonstrated PAM independent behavior of our assembled SMS construct (green) (Figure 1).

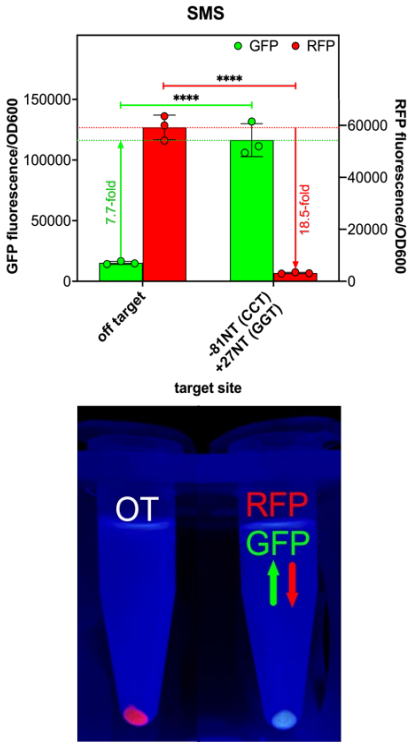


Figure 5: multi gene expression control with SMS

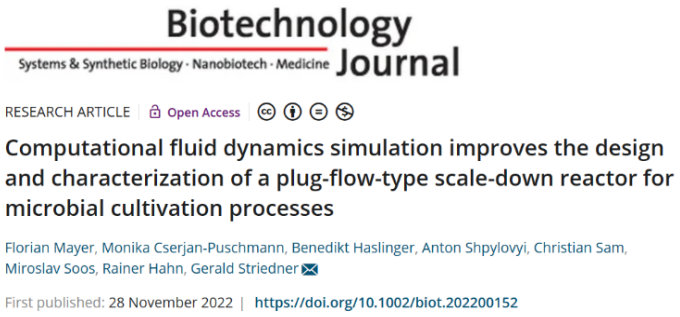
We could also show up-regulation of authentic endogenous genes with SMS at canonical and noncanonical PAM sites, by testing a variety of model genes.

In addition, we demonstrated the abilities of SMS for multi gene expression control. Utilizing two different guide RNAs allowed us to simultaneously up-regulate weak expressed green fluorescent protein (GFP) and down-regulate strong expressed RFP, leading to a remarkable phenotypic switch from red to green cells (Figure 2).

In a nutshell, we established CRISPRa-SMS, a PAM sequence independent up-regulation tool and proved its functionality on various levels. Thus, SMS provides the basis for in-depth genetics studies and sophisticated metabolic engineering approaches in *E. coli* to assist protein production.

References: Dong,C., Fontana,J., Patel,A., Carothers,J.M. and Zalatan,J.G. (2018) Synthetic CRISPR-Cas gene activators for transcriptional reprogramming in bacteria. Nat. Commun., 9, 2489.

Walton,R.T., Christie,K.A., Whittaker,M.N. and Kleinstiver,B.P. (2020) Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants. Science, 368, 290–296.



Biotechnology Journal
Systems & Synthetic Biology · Nanobiotech · Medicine

RESEARCH ARTICLE | Open Access

Computational fluid dynamics simulation improves the design and characterization of a plug-flow-type scale-down reactor for microbial cultivation processes

Florian Mayer, Monika Cserjan-Puschmann, Benedikt Haslinger, Anton Shpylovyi, Christian Sam, Miroslav Soos, Rainer Hahn, Gerald Striedner

First published: 28 November 2022 | https://doi.org/10.1002/biot.202200152

We designed and constructed a two-compartment scale-down bioreactor consisting of a 20 L stirred tank reactor (STR) and a plug-flow reactor (PFR). This device should mimic the heterogenous conditions from production scale reactors of several 1000 L and should help to investigate scale-effects in laboratory scale.

The first task was the design and construction of the scale-down device. The main objectives during this part were the sterilizability, cleanability and flexibility of the device. By using a stainless-steel-setup, we were able to meet all our requirements (Figure 1). Additionally, we used computational fluid dynamics (CFD) simulation to assess the mixing times in a production scale bioreactor and our 20 L laboratory scale bioreactor. The mixing time difference between the scales were used as scale-down criterium for the operation of our STR-PFR scale-down device. The detailed study and results of this task were recently published by Mayer et al. [1].

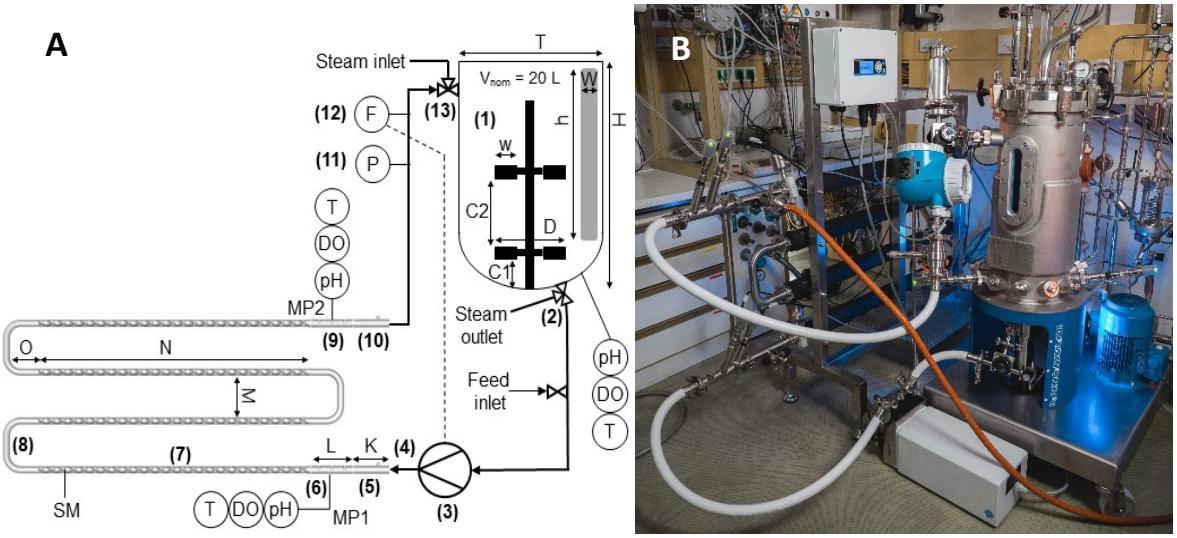
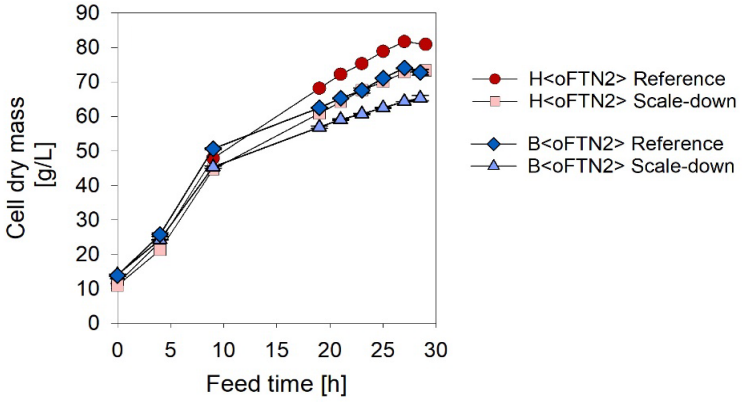


Figure 1: (A) Schematic illustration of the scale-down setup. (B) Scale-down setup in the laboratory. Both pictures are from Mayer et al. [1].

With the scale-down device at hand, we used an industrial relevant, high-cell density, *E. coli* recombinant protein production process, to assess the influence of the scale-effects on the process performance. As model protein we chose an antibody fragment (Fab) and we included two different *E. coli* strains in our study, namely BL21(DE3) and HMS174(DE3). We compared the performance of the two different strains and also their reaction to the scale-effects, with respect to biomass (Figure 2) and product formation. The influences of the scale-effects were assessed by the comparison of a standard laboratory-scale cultivation (reference) with cultivations in our scale-down setup (scale-down). Additionally, we looked at the misincorporation of certain non-canonical amino acids. This

misincorporation is known to increase when cells are exposed to heterogenous conditions, as for example in large scale bioreactors [2]. We found major differences between the strains and between the two different setups. Most mentionable was the difference in non-canonical amino acid misincorporation, where BL21(DE3) did not show this unfavorable effect, whereas HMS174(DE3) did. The results of our study can be found in Mayer et al. [3].

Figure 2: Cell dry mass concentration for the two different strains and different setups. Picture was taken from Mayer et al. [3]



However, we did not only look at upstream relevant parameters of the cultivation process, just as biomass and product formation. We also investigated, how the scale-effects alter fermentation broth parameters relevant for downstream processing. In particular, we were investigating changes in fermentation broth viscosity, particle size distribution and cell morphology. As a result, we were able to detect an increase in cell size and changes in fermentation broth viscosity, when the scale-down setup was used.

In summary, we showed the development of a highly flexible and hygienic scale-down bioreactor, which can be used to simulate heterogenous conditions similar to the conditions in large scale tanks. The use of this device will help to improve process- scale-up in the future. Additionally, we applied an integrated approach, where we did not only investigate the influence of scale-effects on upstream relevant parameters, but also on downstream relevant parameters. This approach can help to improve the entire process chain and not only parts of it.

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Strain specific properties of *Escherichia coli* can prevent non-canonical amino acid misincorporation caused by scale-related process heterogeneities

Florian Mayer, Monika Cserjan-Puschmann, Benedikt Haslinger, Anton Shpylovyi, Thomas Dalik, Christian Sam, Rainer Hahn & Gerald Striedner

Microbial Cell Factories **21**, Article number: 170 (2022) | Cite this article

[1] Mayer, F., Cserjan-Puschmann, M., Haslinger, B., Shpylovyi, A., Sam, C., Soos, M., Hahn, R., & Striedner, G. (2023). Computational fluid dynamics simulation improves the design and characterization of a plug-flow-type scale-down reactor for microbial cultivation processes. Biotechnology Journal, 18, e2200152. https://doi.org/10.1002/biot.202200152

[2] Reitz, C., Fan, Q., Neubauer, P. (2018). Synthesis of non-canonical branched-chain amino acids in Escherichia coli and approaches to avoid their incorporation into recombinant proteins. Current Opinion in Biotechnology, 53, https://doi.org/10.1016/j.copbio.2018.05.003.

[3] Mayer, F., Cserjan-Puschmann, M., Haslinger, B., Shpylovyi, A., Dalik, T., Sam, C., Hahn, R., & Striedner, G. (2022). Strain specific properties of Escherichia coli can prevent non-canonical amino acid misincorporation caused by scale-related process heterogeneities. Microb Cell Fact 21, 170. https://doi.org/10.1186/s12934-022-01895-1

Overview: final theses (finished and ongoing)

PhD projects

Finished

Anna Christler (2022):
Semi-automation of bioanalyses for real-time monitoring of quantity, purity and activity of biopharmaceuticals.
Supervisor: Astrid Dürauer
Finished: February 2022

Carme Pons Royo (ITN CODOBIO)
Millifludic device to accelerate process development
Supervisor: Alois Jungbauer
Finished Dec 09, 2022

Florian Strobl (acib project 25041)
Continuous production of biomolecules with insect cells
Supervisor: Gerald Striedner
Finished: 2022

Sarah Susanne Übleis
Development of vaccine-type specific potency assays.
Supervisor: Karola Vorauer-Uhl
Finished: June 2022

On-Going

Lena Achleitner (acib 94045)
Virus like particle production in insect cells and mammalian cells
Supervisor: Alois Jungbauer
Start: July 2021

Arasteh Kani Arshia (MISTER)
Metabolic incorporation of latent fast reacting thioersters
Supervisor: Birgit Wiltschi, Gerald Striedner
Start: May 2022

Sergio Araujo (FASEP)
Production of Zika surface protein in E. coli
Supervisor: Alois Jungbauer, Viviane Goncalves

Johanna Bacher (acib 94045)
Fundamentals of fast virus quantification
Supervisor: Alois Jungbauer
Start September 2022

Jürgen Beck
Impact of mass transfer mechanism on protein separation in two-component adsorption
Supervisor: Rainer Hahn
Start: November 2019

Markus Berg (acib project 94041)
Model based process development and scale up of primary recovery for biopharmaceutical production
Supervisor: Rainer Hahn, Astrid Dürauer
Start: January 2020

Natalia Danielewicz (enGenes)
Process development for high yield fermentation of active recombinant lectins expressed in Escherichia coli
Supervisor: Gerald Striedner
Start: 2018

Felix Dierlinger (Takeda)
Economic and envrionmetnal modelling of bioprocesses
Supervisor: Alois Jungbauer
Start: October 2021

Gregory Silva Dutra (Marie Curie ITN A4B)
Continuous Separation of Recombinant Antibodies by non-chromatographic methods
Supervisor: Alois Jungbauer
Start: October 2018

Touraj Eslami (H2020 ITN CODOBIO, evon)
Online control of chromatographic steps using model predictive control (MPC) in continuous downstream processing
Supervisor: Gerald Ebner, Alois Jungbauer, Nico Lingg
Start: 2019

Anna-Carina Frank (NextFlock)
Flocculants for improved monoclonal antibody purification.
Supervisor: Rainer Hahn, Peter Satzer
Start: June 2022

Lisa Fohler (ENZYCLE)
Fed-batch and continuous production of PET degrading enzymes in E. coli
Supervisor: Gerald Striedner
Start: July 2021

Nils Gehrmann (Sartorius)
An ultrafast antibody purification process based on membrane chromatography
Supervisor: Rainer Hahn
Start: October 2020

Martin Gibisch (CD-Lab NLBP)
Directed evolution using selective advantage for producing cells
Supervisor: Monika Cserjan, Gerald Striedner

Start: December 2020

Stephan Gutmann (CD-Lab NLBP)
Directed evolution using selective advantage for producing cells
Supervisor: Monika Cserjan, Reingard Grabherr, Gerald Striedner
Start: December 2020

Hanna Hanee Ahvaz (CD-Lab NLBP)
Development of Methods for in vivo quantification of proteolysis in E. coli expression systems
Supervisor: Gerald Striedner
Start: November 2019

Leo Jakob (BioTop)
Protein solubility in buffers with kosmotropic salts and polyols
Supervisor: Alois Jungbauer, Nico Lingg, Rupert Tscheließnig
Start: Juni 2019

Alexander Jurjevec (CD-Lab NLBP)
Polyethyleneimmine for protein extraction from bacteria
Supervisor: Rainer Hahn
Start: 2019

Christoph Köppl (acib project 94081)
Fusion Tag design for generic CASPON platform
Supervisor: Monika Cserjan, Gerald Striedner
Start: Oktober 2020

Marco Kreß (Valneva)
Fast virus manufacturing
Supervisor: Alois Jungbauer
Start: June 2019

Claudia Lacombe (H2020 Fet Open PURE)
Production of ncAAs for spider silk protein modification
Supervisor: Birgit Wiltschi, Gerald Striedner
Start: November 2020

Narges Lali (H2020 ITN CODOBIO)
Residence Time distribution of pseudo-continuous methods
Supervisor: Alois Jungbauer
Start: June 2019

Zana Marin (synPPP)
Synthetic in Pichia pastoris
Supervisor: Birgit Wiltschi, Gerald Striedner
Start: January 2022

Florian Mayer (CD-Lab NLBP)
Influence of fermentation strategies and scale effects on Fab production in E. coli
Supervisor: Gerald Striedner
Start: July 2019

Victoria Mayer (acib)
Characterization of bionanoparticles by biophysical methods
Supervisor: Alois Jungbauer and Patricia Aguilar
Start: October 2020

Matthias Müller (CD-Lab NLBP)
Investigation on disulfide bridge formation in peptides produced by periplasmic expression in Escherichia coli
Supervisor: Rainer Hahn
Start: Februar 2021

Ignacio Montes Serrano (H2020 ITN CODOBIO)
Determination of a mathematical model for the power input in shaken microtiter plates and correlation with larger size vessels
Supervisor: Astrid Dürauer
Start: 2019

Bettina Motycka (BioTop)
Resolving dynamic protein conformations in multidomain enzymes with SAXS
Supervisors: Roland Ludwig, Rupert Tscheliessnig
Start: 2019

Magdalena Pappenreiter (FFG Cubic)
Development of perfusion control concepts and integrated solutions in fully continuous and automated End-to-End biomanufacturing processes
Supervisor: Alois Jungbauer
Start: January 2020

Gabriele Recanati (FFG Cubic)
Modeling, optimization and automation of a continuous manufacturing process platform for biopharmaceuticals focused on downstream unit operations
Supervisor: Alois Jungbauer
Start: November 2020

Tommaso de Santis (ENZYCLE)
Economic modeling of enzyme based plastics degradation and recycling processes
Supervisor: Gerald Striedner
Start: December 2020

Mafalda dos Santos (acib 91201)
Nanomembranes for in-situ removal of products from fermentation broth
Supervisor: Alois Jungbauer
Start: November 2021

Sonja Schürer-Waldheim (BioTop)
Phosphoproteomics of antibody producing CHO cell lines
Supervisors: Renate Kunert, Gorji Marzban
Start: 2019

Sophie Anna Vazulka (CD-Lab NLBP)
Host cell response to antibody fragment production in E. coli with special focus on transcriptome and translatome
Supervisor: Gerald Striedner
Start: January 2019

Yao Yuelang (Biotop Joint Project BOKU)
Effect of membrane organization on weak acid transport proteins;
Supervisor: Vorauer-Uhl Karola and Michael Sauer
Start: August 2020

Alexander Zollner (H2020 Fet Open PURE)
Functionalized non-woven nanofibers for the purification of virus-like particles
Supervisor: Alois Jungbauer
Start: September 2021

Master theses

Finished

Marina Baumann (CD-Lab NLBP)
Production of antibody fragments with site specific incorporated non-canonical amino acids – evaluation of a modified E. coli expression host
Supervisor: Gerald Striedner, Hana Hanaee, Dana Mezricky (FH Krems)
Finished: October 2022

Hugo Filipe Quaresma Val-Flores França (CD-Lab NLBP)
Influence of Lpp and LolA downregulation on recombinant peptide production and outer membrane permeability in E.coli
Supervisor: Gerald Striedner, Martin Gibisch, Miguel Nobre Parreira Cacho Teixeira (Univ. de Lisboa)
Finished: December 2022

Benedikt Haslinger (CD-Lab NLBP)
Fermentation scale effects on downstream processability of E. coli fermentation broths
Supervisor: Gerald Striedner, Florian Mayer
Finished: June 2022

Marco Kaupe (BioIndustrial Pilot Plant)
Risk-based quality assurance for process control system operations in a multipurpose Pilot Plant
Supervisor: Karola Vorauer-Uhl, Markus Luchner
Finished: July 2022

Konstanze Kastenhofer (AGES)
Brivaracetam – practical considerations for the development of a European Pharmacopoeia Monograph
Supervisor: Karola Vorauer-Uhl
Finished: October 2022

Roman Liebhart (CD-Lab NLBP)
Balancing heavy and light chain expression to increase correctly folded Fab production in Escherichia coli.
Supervisor: Gerald Striedner, Monika Cserjan
Finished: August 2022

Alexander Mechtler
Investigations on solid diffusion mass transfer on anion exchange chromatography resins
Supervisor: Rainer Hahn
Finished: October 2022

Markus Mozgovicz (acib project 91023)
Adsorption effects on thermal stability of proteins
Supervisor: Alois Jungbauer, Nico Lingg
Finished: 2022

David Scheich
Experimental characterization and modelling of flow non-uniformities in small chromatography columns
Supervisor: Rainer Hahn
Finished: December 2022

Anton Shpylovyi (CD-Lab NLBP)
Fermentation scale effects on product related non-canonical amino acid misincorporation in different E. coli strains
Supervisor: Gerald Striedner, Monika Cserjan
Finished: November 2022

Florian Simon (CD-Lab NLBP)
Influence of MicL co-expression on growth and periplasmic sfGFP production kinetics in Escherichia coli lab-scale bioreactor cultivations
Supervisor: Gerald Striedner, Monika Cserjan
Finished: April 2022

Lina Vranizky (RSA Novasign)
Intensification of experimental design for Escherichia coli fed-batch fermentation
Supervisor: Gerald Striedner, Bayer Benjamin
Finished: November 2022

On-going

Johanna Berein (enGenes GmbH)
Implementing growth-decoulped recombinant protein production in E. coli K-12 strains
Supervisor: Gerald Striedner, Jürgen Mairhofer
Start: December 2021

Nora Dürkop (BIOMIN)
Optimization of the drying process of a bacterial feed additive
Supervisor: Gerald Striedner
Start: July 2019

Felix Oliver Faschingeder (CD-Lab NLBP)
Recombinase mediated cassette exchange (RMCE) in E. coli
Supervisor: Gerald Striedner, Gutmann Stefan
Start: October 2022

Pawel Gorecki (CD-Lab NLBP)
Outer membrane permeabilization for extracellular recombinant peptide production in E. coli
Supervisor: Gerald Striedner, Monika Cserjan
Start: December 2022

Marina Heine (Takeda)
Downscale model qualification on example of plasma-derived serine protease inhibitor purification process
Supervisor: Alois Jungbauer
Start: Mar 2020

Georg Hochdaninger
Separation of two-component mixture of Conalbumin and Green Fluorescent Protein
Supervisor: Rainer Hahn
Start: May 2022

Lea Jusufagic (CD-Lab NLBP)
CRISPRactivation mediating chaperon upregulation enhances recombinant polypeptide production in E. coli
Supervisor: Gerald Striedner, Monika Cserjan

Tobias Kargl (FFG Research Studio Novasign)
Predictive Hybrid Modeling of Single-Pass Tangential Flow Filtration
Supervisor: Astrid Dürauer
Start: 2020

Lukas Leibetseder (Enzycle)
Production of polyolefine degrading enzymes with E. coli expression systems and initial activity/degradation testing
Supervisor: Gerald Striedner
Start: October 2022

Lea Milas (Takeda)
AAV purification by affinity chromatography
Supervisor: Astrid Dürauer
Start 09/2022

Franz Moisi (Valneva)
Evaluation of fermentation process parameters influencing the fatty acid composition of bacterial lipoprotein
Supervisor: Gerald Striedner
Start: July 2018

Dieter Ratz
Downstream processing of a membrane bound Cytochrome P450 expressed in Pichia pastoris
Supervisor: Rainer Hahn, Diana Huber (Technical University Vienna)
Start: June 2022

Lukas Richter (Biomay)
Comparative Study of Chromatographic purification processes for mRNA manufacturing
Supervisor: Astrid Dürauer
Start 05/2022

Michaela Stadlmayr (Biomay)
Development of fast and robust purification process for plasmid DNA
Supervisor: Astrid Dürauer
Start 05/2022

Martina Christine Winter (research project)
Soft sensor for determining oxygen consumption rate and viable cell count
Supervisor: Satzer Peter, Gerald Striedner
Start: August 2022

Bachelor theses

Finished

Dominik Kallinger
Bacterial Cell disruption in a Bench-top Bead Mill
Supervisor: Astrid Dürauer
Start: January 2022

Alexandra Katholnig (HIV-Vaccine)
Optimization of N-terminal reductive alkylation of proteins for bioconjugation to liposomes;
Supervisor: Karola Vorauer-Uhl
Start: September 2019

On-going

Karoline Reznar (CD-Lab NLBP)
Downregulation of the periplasmic chaperon LolA by a synthetic small regulatory RNA strategy to increase cell permeability in Escherichia coli
Supervisor: Gerald Striedner, Monika Cserjan
Start: August 2021

Scientific output

Scientific publications in peer-reviewed journals

Bayer, B; Maccani, A; Jahn, J; Duerkop, M; Kapeller, E; Pletzenauer, R; Kraus, B; Striedner, G; Hernandez Bort, JA; Proton-transfer-reaction mass spectrometry (PTR-MS) for online monitoring of glucose depletion and cell concentrations in HEK 293 gene therapy processes. Biotechnol Lett. 2022; 44(1):77-88; DOI: 10.1007/s10529-021-03205-y.

Berg, M.C., Beck, J., Karner, A., Holzer, K., Dürauer, A., Hahn, R. Mass transfer of proteins in chromatographic media: Comparison of pure and crude feed solutions. (2022) Journal of Chromatography A, 1676, 463264, DOI: 10.1016/j.chroma.2022.463264

Berg, MC; Beck, J; Karner, A; Holzer, K; Dürauer, A; Hahn, R; Mass transfer of proteins in chromatographic media: Comparison of pure and crude feed solutions..J Chromatogr A. 2022; 1676:46326

Danielewicz N, Dai W, Rosato F, Webb ME, Striedner G, Römer W, Turnbull WB, Mairhofer J. In-Depth Characterization of a Re-Engineered Cholera Toxin Manufacturing Process Using Growth-Decoupled Production in Escherichia coli. Toxins (Basel). 2022 Jun 8;14(6):396. doi: 10.3390/toxins14060396.

Eslami, T; Jakob, LA; Satzer, P; Ebner, G; Jungbauer, A; Lingg, N. Productivity for free: Residence time gradients during loading increase dynamic binding capacity and productivity. SEP PURIF TECHNOL. 2022; 281, 119985

Eslami, T; Steinberger, M; Csizmazia, C; Jungbauer, A; Lingg, N. Online optimization of dynamic binding capacity and productivity by model predictive control. J CHROMATOGR A. 2022; 1680, 463420

Jakob LA, Mesurado T, Jungbauer A, Lingg N. Increase in cysteine-mediated multimerization under attractive protein-protein interactions. Prep Biochem Biotechnol. 2022 Dec 28:1-15. doi: 10.1080/10826068.2022.2158471.

Jakob LA, Mesurado T, Jungbauer A, Lingg N. Increase in cysteine-mediated multimerization under attractive protein-protein interactions. Prep Biochem Biotechnol. 2022 Dec 28:1-15. doi: 10.1080/10826068.2022.2158471.

Jiang Q, Seth S, Scharl T, Schroeder T, Jungbauer A, Dimartino S. Prediction of the performance of pre-packed purification columns through machine learning. J Sep Sci. 2022 Apr;45(8):1445-1457. doi: 10.1002/jssc.202100864.

Klanschnig M, Cserjan-Puschmann M, Striedner G, Grabherr R. CRISPRactivation-SMS, a message for PAM sequence independent gene up-regulation in Escherichia coli. Nucleic Acids Res. 2022 Oct 14;50(18):10772-10784. doi: 10.1093/nar/gkac804.

Kopp J, Bayer B, Slouka C, Striedner G, Dürkop M, Spadiut O. Fundamental insights in early-stage inclusion body formation. Microb Biotechnol. 2022 Jul 13. doi: 10.1111/1751-7915.14117.

Köppl C, Lingg N, Fischer A, Kröß C, Loibl J, Buchinger W, Schneider R, Jungbauer A, Striedner G, Cserjan-Puschmann M. Fusion Tag Design Influences Soluble Recombinant Protein Production in Escherichia coli. Int J Mol Sci. 2022 Jul 12;23(14):7678. doi: 10.3390/ijms23147678.

Lali N, Satzer P, Jungbauer A. Residence time distribution in counter-current protein A affinity chromatography using an inert tracer. J Chromatogr A. 2022 Nov 8;1683:463530. doi: 10.1016 /j.chroma.2022.463530.

Lali, N. , Jungbauer, A., Satzer, P.; Traceability of products and guide for batch definition in integrated continuous biomanufacturing, Journal of Chemical Technology and Biotechnology, 2022, 97(9), pp. 2386–2392

Lingg N, Kröß C, Engele P, Öhlknecht C, Köppl C, Fischer A, Lier B, Loibl J, Sprenger B, Liu J, Scheidl P, Berkemeyer M, Buchinger W, Brocard C, Striedner G, Oostenbrink C, Schneider R, Jungbauer A, Cserjan-Puschmann M.

CASPON platform technology: Ultrafast circularly permuted caspase-2 cleaves tagged fusion proteins before all 20 natural amino acids at the N-terminus. N Biotechnol. 2022 Nov 25;71:37-46. doi: 10.1016/j.nbt.2022.07.002.

Lingg, N., Daxbacher, A., Womser-Matlschweiger, D., Pum, D., Beck, J., Hahn, R. Alkaline treatment enhances mass transfer in Protein A affinity chromatography. (2022) Journal of Chromatography A, 1673, 463058, DOI: 10.1016/j.chroma.2022.463058

Mayer F, Cserjan-Puschmann M, Haslinger B, Shpylovyi A, Dalik T, Sam C, Hahn R, Striedner G. Strain specific properties of Escherichia coli can prevent non-canonical amino acid misincorporation caused by scale-related process heterogeneities. Microb Cell Fact. 2022 Aug 23;21(1):170. doi: 10.1186/s12934-022-01895-1.

Mayer F, Cserjan-Puschmann M, Haslinger B, Shpylovyi A, Sam C, Soos M, Hahn R, Striedner G. Computational fluid dynamics simulation improves the design and characterization of a plug-flow-type scale-down reactor for microbial cultivation processes. Biotechnol J. 2023 Jan;18(1):e2200152. doi: 10.1002/biot.202200152.

Mayer, F., Cserjan-Puschmann, M., Haslinger, B., Shpylovyi, A., Dalik, T., Sam, C., Hahn, R., Striedner, G. Strain specific properties of Escherichia coli can prevent non-canonical amino acid misincorporation caused by scale-related process heterogeneities. (2022) Microbial cell factories, 21 (1), p. 170. DOI: 10.1186/s12934-022-01895-1

Montes-Serrano I, Satzer P, Jungbauer A, Dürauer A. Characterization of hydrodynamics and volumetric power input in microtiter plates for the scale-up of downstream operations. Biotechnol Bioeng. 2022 Feb;119(2):523-534. doi: 10.1002/bit.27983.

Montes-Serrano, I; Satzer, P; Jungbauer, A; Dürauer, A, Characterization of hydrodynamics and volumetric power input in microtiter plates for the scale-up of downstream operations. BIOTECHNOL BIOENG. 2022; 119(2): 523-534.

Pappenreiter M, Döbele S, Striedner G, Jungbauer A, Sissolak B. Model predictive control for steady-state performance in integrated continuous bioprocesses. Bioprocess Biosyst Eng. 2022 Sep;45(9):1499-1513. doi: 10.1007/s00449-022-02759-z.

Pinto, J; Mestre, M; Ramos, J; Costa, RS, Striedner; G; Oliveira, R. A general deep hybrid model for bioreactor systems: Combining first principles with deep neural networks. Computers & Chemical Engineering, Volume 165, September 2022, 107952; https://doi.org/10.1016/j.compchemeng.2022.107952

Pons Royo, M.D.C., Beulay, J.-L., Valery, E., Jungbauer, A., Satzer, P.; Design of millidevices to expedite apparent solubility measurements. Reaction Chemistry and Engineering, 2022, 7(9), pp. 2045–2053

Pons Royo, M.D.C., Beulay, J.-L., Valery, E., Jungbauer, A., Satzer, P.; Mode and dosage time in polyethylene glycol precipitation process influences protein precipitate size and filterability. Process Biochemistry, 2022, 114, pp. 77–85

Pons Royo, M.D.C., Montes-Serrano, I., Valery, E., Jungbauer, A., Satzer, P.; Milliscale reactors for integration of continuous precipitation and filtration. Journal of Chemical Technology and Biotechnology, 2022, 97(11), pp. 3183–3192

Puente-Massaguer, E; Gonzalez-Dominguez, I; Strobl, F; Grabherr, R; Striedner, G; Lecina, M; Godia, F. Bioprocess characterization of virus-like particle production with the insect cell baculovirus expression system at nanoparticle level. J CHEM TECHNOL BIOT. 2022; 97(9): 2456-2465. https://doi.org/10.1002/jctb.7105.

Satzer, P., Achleitner, L.; 3D printing: Economical and supply chain independent single-use plasticware for cell culture. New Biotechnology. 2022, 69, pp. 55–61

Satzer, P., Komuczki, D., Pappenreiter, M., Cataldo, L., Sissolak, B., Jungbauer, A.; Impact of failure rates, lot definitions and scheduling of upstream processes on the productivity of continuous integrated bioprocesses. Journal of Chemical Technology and Biotechnology, 2022, 97(9), pp. 2393–2403

Steppert P, Mosor M, Stanek L, Burgstaller D, Palmberger D, Preinsperger S, Pereira Aguilar P, Müllner M, Csar P, Jungbauer A. A scalable, integrated downstream process for production of a recombinant measles virus-vectored vaccine. Vaccine. 2022 Feb 23;40(9):1323-1333. doi: 10.1016/j.vaccine.2022.01.004.

Sun YN, Shi C, Zhong XZ, Chen XJ, Chen R, Zhang QL, Yao SJ, Jungbauer A, Lin DQ. Model-based evaluation and model-free strategy for process development of three-column periodic counter-current chromatography. J Chromatogr A. 2022 Aug 16;1677:463311. doi: 10.1016/j.chroma.2022.463311.

Vazulka S, Schiavinato M, Wagenknecht M, Cserjan-Puschmann M, Striedner G. Interaction of Periplasmic Fab Production and Intracellular Redox Balance in Escherichia coli Affects Product Yield. ACS Synth Biol. 2022 Feb 18;11(2):820-834. doi: 10.1021/acssynbio.1c00502.

Presentations and Posters

Beck, J; Carta, G; Hahn, R. Patterns of Protein Adsorption in Ion-exchange Particles and Columns Predicted for Pore and Solid Diffusion Mechanisms. 35th PREP Symposium, 15.05.2022-18.05.2022, Baltimore, USA.

Beck, J; Hahn, R. Impact of adsorbed phase protein mobility on chromatographic separation performance. International Chemical Engineering Symposia, 16.03.2022 - 18.03.2022, JAPAN (Virtual Conference)

Beck, J; Hochdaninger, G; Hahn, R. Impact of resin architecture on intraparticle mass transport in a two component system. Biopartitioning and Purification Conference, 25.09.2022 - 28.09.2022, Aveiro, PORTUGAL

Berg, MC; Hahn, R; Dürauer, A (2022): Mass transfer of proteins in chromatographic media: Comparison of pure and crude feed solutions. Biopartitioning and Purification Conference 2022 (BPP2022), 25.09.2022-28.09.2022, Aveiro, PORTUGAL

Berg, MC; Hahn, R; Dürauer, A. Mass transfer of proteins in chromatographic media: Comparison of pure and crude feed solutions. Biopartitioning and Purification Conference 2022 (BPP2022), 25.09.2022-28.09.2022, Aveiro, PORTUGAL

Berg, MC; Hahn, R; Dürauer, A. Mass transfer of proteins in chromatographic media: Comparison of pure and crude feed solutions. European Summit of Industrial Biotechnology (esib2022), 14.11.2022-16.11.2022, Graz, AUSTRIA

Berg, MC; Hahn, R; Dürauer, A; (2022): Mass transfer of proteins in chromatographic media: Comparison of pure and crude feed solutions. [Poster] European Summit of Industrial Biotechnology (esib2022), 14.11.2022-16.11.2022, Graz, AUSTRIA

Cserjan-Puschmann, M; Köppl, C; Lingg, N; Fischer A; Krößß, C; Schneider, R; Jungbauer, A; Striedner, G; (2022): CASPON technology – a platform process for non-platform proteins using Escherichia coli. 13th ESBES Symposium 2022 - (Bio)Process Engineering - a Key to Sustainable Development, 12.09.2022 - 15.09.2022, Aachen, GERMANY

Dürauer, A (2022): Predictive Models for Process Development and Multidimensional Real Time Monitoring in Downstream Processing . Recovery of Biological Products XIX, 10.07.2022-15.07.2022, Rome, Italy

Gehrmann, N; Desch, F; Toeppner, K; Taft, F; Hahn, R; Thom, V. A Capture Sep in Antibody Purification using a Novel Protein A Coupled Membrane Adsorber Prototype. 35th PREP Symposium, 15.05.2022-18.05.2022, Baltimore, USA.

Gutmann Stephan (2022): Addiction systems make targeted evolution achievable for continuous cultivation of *Escherichia coli* producing recombinant proteins. ÖGMBT 14th Annual Meeting, 19.11.2022 - 23.11.2022, Vienna.

Hahn, R. Protein A Affinity Chromatography: Past, Present; Future. Recovery of Biological Products RXIX, 10.07.2022-15.07.2022, Rom, ITALY

Jurjevec, A; Cserjan-Puschmann, M; Brocard, C; Striedner, G; Hahn, R. Multicomponent adsorption as hindrance of Fab purification. Biopartitioning and purification conference, 25.09.2022 - 28.09.2022, Aveiro, PORTUGAL

Jurjevec, A; Cserjan-Puschmann, M; Brocard, C; Striedner, G; Hahn, R (2022): Multicomponent adsorption as hindrance of Fab purification. Biopartitioning and purification conference, 25.09.2022 - 28.09.2022, Aveiro, Portugal

Koppl, C; Cserjan-Puschmann, M; Lingg, N; Striedner, G (2022): Combinatorial Fusion TAG yields powerful platform process for the production of pharmaceutically relevant proteins. Microbial Engineering II, Albufeira, PORTUGAL, 03.04.2022 - 07.04.2022

Köppl, C; Cserjan-Puschmann, M; Lingg, N; Striedner, G. (2022): Bacteriophage derived expression enhancing tag yields powerful platform process for the production of recombinant fusion proteins. 2nd Edition of Euro-Global Conference on Biotechnology and Bioengineering, 13.06.2022 - 14.06.2022, virtuell

Lingg, N., Beck, J., Hahn, R. (2022): Mass transfer changes in protein A affinity chromatography after alkaline treatment. 35th International Symposium on Preparative and Process Chromatography, 15.05.2022 - 18.05.2022, Baltimore, USA

Lingg, N; Fischer, A; Jungbauer, A. (2022): CASPON – a platform process for non-platform proteins. 35th International Symposium on Preparative and Process Chromatography, 15.05.2022 - 18.05.2022, Baltimore, USA

Lingg, N; Mozgovicz, M; Tscheliessnig, AL; Jungbauer, A. (2022): Kinetic effects of protein fouling in chromatography. 41st International Symposium and Exhibit on the Separation, Purification, and Characterization of Biologicallty Relevant Molecules (ISPPP), 16.10.2022-19.10.2022, Delray Beach, USA

Lingg, N; Pereira Aguilar, P; Hochstein, R; Voloshin, A; Jungbauer; A. (2022): Virus and virus like particle purification by fiber chromatography. ACS Spring 2022, MAR 20-24, 2022, San Diego, USA

Lingg, N; Pereira Aguilar, P; Jungbauer, A. (2022): Virus and Virus-Like Particle Purification by Fiber Chromatography. 41st International Symposium and Exhibit on the Separation, Purification, and Characterization of Biologicallty Relevant Molecules (ISPPP), 16.10.2022-19.10.2022, Delray Beach, USA

Matthias Medl, Theresa Scharl, Astrid Dürauer, Friedrich Leisch (2022): Deep Learning for Modelling High-dimensional Data of a Biopharmaceutical Purification Process. Austrian and Solvenian Statistical Days 2022, 20.04.2022-22.04.2022, Graz

Matthias Medl, Theresa Scharl, Astrid Dürauer, Friedrich Leisch (2022): Deep learning to predict critical process parameters of an antibody capture process . 14th OEGMBT Annual Meeting, 20.09.2022 - 22.09-2022, Wien

Matthias Medl, Theresa Scharl, Astrid Dürauer, Friedrich Leisch (2022): Permutation based variable importance determination for deep learning . COMPSTAT22 - International Conference on Computational Statistics, 23.08.22-26.08.22, Bologna

Matthias Medl, Theresa Scharl, Astrid Dürauer, Friedrich Leisch (2022): Time Resolved Feature Importance of a Biopharmaceutical Purification Process Using Permutation Based Methods. IFCS22 - Classification and Data Science in the Digital Age , 19.07.2022-23.07.2022, Porto

Mayer, F; Cserjan-Puschmann, M; Sam, C; Soos, M; Striedner, G. (2022): Integrated processing meets scale-down. (Bio)Process Engineering – a Key to Sustainable Development, 12.09.2022 - 15.09.2022, Aachen, GERMANY

Müller, M; Gibisch, M; Brocard, C; Cserjan, M; Hahn, R. Extraction and purification of recombinantly produced disulfide bond containing peptides. Biopartitioning and purification conference - BPP22, 25.09.2022 - 28.09.2022, Aveiro, PORTUGAL

Scheich, D; Rao, J; Beck, J; von Lieres, E; Hahn, R. Experimental Characterization And Modelling Of Flow Non-uniformities In Small Chromatography Columns. 35th PREP Symposium, 15.05.2022-18.05.2022, Baltimore, USA.

Striedner Gerald (2022): Continuous Production with E. coli. 21st Annual PepTalk Conference, 17.01.2022 - 19.01.2022, San Diego

Striedner Gerald (2022): Intensified and Continuous Processing. Bioprocessing Summit Europe 2022, 14.03.2022 - 16.03.2022, Barcelona

Striedner Gerald (2022): Production of High-Quality Plasmid DNA: A Key Ingredient in Vaccine Production Processes. Bioprocessing Summit Boston 2022, 15.08.2022 - 18.08.2022, Boston

Poster Presentations

Fohler, L.; Striedner, G.; Cserjan, M. (2022): mbDOE development of optimized fed-batch cultivation for the production of PET degrading enzymes in E. coli. 6th Applied Synthetic Biology in Europe (ASBE VI), 02.-04.11.2022, Edinburgh.

Fohler, L.; Striedner, G.; Cserjan, M. (2022): mbDOE for development of optimized fed-batch cultivation for the production of PET degrading enzymes in E. coli. Microbial Engineering II, 03.-07.04.2022, Albufeira

Hana Hanaee-Ahvaz, Monika Cserjan-Puschmann, Christopher Tauer, Gerald Striedner. (2022): Monitoring the stability and function of non-canonical amino acid incorporated antibody fragments produced in a plasmid-based E. coli expression system. ESBES, Aachen, Germany, 12.09.2022-15.09.2022

Köppl, C; Cserjan-Puschmann, M; Lingg, N; Striedner, G. (2022): Bacteriophage derived expression enhancing tag yields powerful platform process for the production of recombinant fusion proteins. ESBES2022 — 13th European Symposium on Biochemical Engineering Sciences, 12.09.2022 - 15.09.2022, Aachen, Germany

Köppl, C; Cserjan-Puschmann, M; Lingg, N; Striedner, G. (2022): Bacteriophage derived expression enhancing tag yields powerful platform process for the production of recombinant fusion proteins. European Summit of Industrial Biotechnology, 14.11.2022 - 16.11.2022, Graz, Austria

Marco Klanschnig, Monika Cserjan-Puschmann, Gerald Striedner, Reingard Grabherr. (2022): CRISPRactivation-SMS, a message for PAM sequence independent gene up-regulation in Escherichia coli. [5th International Conference on CRISPR Technologies, University of California, Berkeley, 31.10.2022-02.11.2022

Marco Klanschnig, Monika Cserjan-Puschmann, Gerald Striedner, Reingard Grabherr. (2022) CRISPRactivation-SMS, a message for PAM sequence independent gene up-regulation in Escherichia coli. Genome Engineering: CRISPR Frontiers, Cold Spring Harbor Laboratory, New York, 24.08.2022-27.08.2022

Martin Gibisch, Matthias Müller, Christopher Tauer, Hugo Franca, Rainer Hahn, Monika Cserjan-Puschmann, Gerald Striedner (2022): Adjusting membrane permeability for extracellular peptide production in growing Escherichia coli. Biopartioning and purification, 25.09-28.09.22, Aveiro

Mayer, F; Cserjan-Puschmann, M; Sam, C; Soos, M; Striedner, G. (2022): Integrated processing meets scale-down. Himmelfahrtstagung on Bioprocess Engineering – Future Bioprocesses for a Sustainable Industry, 23.05.2022 - 25.05.2022, Mainz, GERMANY

Mayer, F; Cserjan-Puschmann, M; Sam, C; Soos, M; Striedner, G. (2022): Scale-down of high cell density Fab production in E. coli. 7th BioProScale Symposium, 28.03.2022 - 31.03.2022, Berlin, GERMANY

S. Gutmann, M. Cserjan-Puschmann, M. Wagenknecht, R. Grabherr, G. Striedner (2022): Targeted evolution for continuous production in *Escherichia coli*. 6th Applied Synthetic Biology in Europe, 02.11.2022 -04.11.2022, Edinburg.

Other publications

Book chapters

Three book chapters in Methods in Molecular Biology: "Plant Functional Genomics", Springer Nature.

1D Electrophoresis of plant samples, Gorji Marzban & Donatella Tesei

2D Electrophoresis of plant samples, Gorji Marzban & Donatella Tesei

Plant Phosphoproteomics, Gorji Marzban & Eldi Sulaj

Guest Editorial Activity

Special Issue "Multi-Omics of Extremophilic Organisms" A special issue of *Biology*, MDPI (ISSN 2079-7737). This special issue belongs to the section "Microbiology". Guest editors: Gorji Marzban & Donatella Tesei.

https://www.mdpi.com/journal/biology/special_issues/6793MYHB39.

Teaching activities

#	Title	Programme	ECTS
166655	Integrated biopharmaceutical production in pilot scale	TU Vienna	6
772327	Biochemical and biotechnological methods (analytics design) (in Eng.)	BT	3
790044	Sicherheit am Arbeitsplatz	Bachelor´s FBT	2
790049	Masterseminar Angewandte Mikrobiologie (in Eng.)	Master´s FBT	2
790105	Practical course in applied microbiology	FBT	4
790107	Bachelor's thesis seminar	Bachelor´s FBT	12
790120	Grundlagen der Bioverfahrenstechnik	Bachelor´s FBT	5.5
790321	Biotechnol. Praktikum	Master´s FBT	4.5
790350	Bioprocess engineering I (in Eng.)	BT	3
790353	Quality management in biotechnology (in Eng.)	BT	3
790358	Bioprocess engineering II (in Eng.)	BT	3
790359	Bioprocess engineering laboratory (in Eng.)	BT	5
790371	Automation of bioprocesses (in Eng.)	BT	2
790380	Engineering of biotechnological production facilities (in Eng.)	BT	2
790419	Journal club BioToP III (in Eng.)	DK BioToP	1.5
790423	Doctoral seminar BioToP III (in Eng.)	DK BioToP	1.5
790431	Pilot plant BioproEng (in Eng.)	DK BPE	8
790432	Doctoral Seminar BPE	DK BPE	0.5
790433	Journal Club BPE	DK BPE	0.5
790438	Biothermodynamics (in Eng.)	DK BPE	2
790940	Dissertantenseminar aus Angewandte Mikrobiologie	BT	2
791432	Doctoral seminar BioproEng I (in Eng.)	DK BIOTOP	0.5
791433	Journal club BioproEng I (in Eng.)	DK BPE	3
791437	Automation and control in laboratory (in Eng.)	DK BPE	2
791438	Biothermodynamics (in Eng.)	DK BPE	2
894404	Basic course IV - bioinformatics and molecular modelling (in Eng.)	DK BIOTOP	3
894415	Instructional course IVA - molecular modelling (in Eng.)	DK BIOTOP	3

BT … Biotechnology, FBT … food and biotechnology, DK BPE … Doctoral School Bioprocess Engineering,

External Teaching Activities and Courses 2022

Organization	Title	Program
FH-Bioengineering, Campus Wien	Qualitätskontrolle	Master Quality Management
FH-Bioengineering, Campus Wien	Qualitätskontrolle und Qualitätssicherung im Prüflaboratorium	Master Quality Management
FH-Bioengineering, Campus Wien	Downstream Processing, Protein VO, Downstream Processing Labor UE, Gärungstechnisches Labor UE	Bachelor Bioengineering
FH-Bioengineering, Campus Wien	Downstream Processing, Protein VO+UE	Master Biotechnology
IMC FH Krems	Process Control and Process Online Monitoring	Master Biotechnology
Montan Universität Leoben	Qualitätssicherung im chemischen Labor	University Course

Epilogue and outlook

The annual report serves to present the achievements in our core tasks of science, teaching and project acquisition. The compilation of the achievements and the selected highlights in the report illustrates that the IBSE team has been very active and successful in 2022. However, we want to take the chance to explicitly mention the workload of administration and organization which is not given in numbers in the annual report. The development of the last few years clearly showed that these tasks, which are regarded as incidental, are becoming increasingly burdensome for all employees. We can only manage these tasks thanks to the great effort of our team assistant Petra Polak, mainly financed by IBSE third-party funds, and the excellent support of the DBT administrative office.

2023 is going to be an exciting year for IBSE with many research projects planned, project funding and teaching. The new *CD Laboratory for Knowledge-Based Production of Gene Therapy Vectors*, headed by Astrid Dürauer will start in January and the already assembled research team will address this scientific topic on fundamental and applied research levels.

2023 is also the year when we will apply for the extension of the ACIB FFG COMET K2 program. IBSE is involved in the intensive discussions on research projects with our academic and industrial partners in different thematic areas and will participate accordingly in the design of the application to finally achieve a joint success.

In the area of teaching, work is focused on the redesign of the bachelor's degree program in food and biotechnology. IBSE members are involved in this process in the relevant committees, and we are very optimistic that we will be able to develop a bachelor's program of high quality and improved studyability.

With this, we are pleased to present IBSE's achievements in 2022 and look forward to the news and results that will be presented in the next report.

Gerald Striedner Astrid Dürauer

On behalf of the entire IBSE Team

Contact

Postal address (German/English)

Universität für Bodenkultur
Department für Biotechnologie
Institut für Bioverfahrenstechnik
Muthgasse 18
1190 Wien
Österreich

University of Natural Resources and Life Sciences, Vienna
Department of Biotechnology
Institute of Bioprocess Science and Engineering
Muthgasse 18
1190 Vienna,
Austria

Homepage

https://boku.ac.at/dbt/ibse

Head of the *Institute of Bioprocess Science and Engineering*

Univ.-Prof. Dipl.-Ing. Dr. Gerald Striedner
gerald.striedner@boku.ac.at
0043 1 47654-79077

Deputy of the *Institute of Bioprocess Science and Engineering*

Priv.-Doz. Dipl.-Ing. Dr. Astrid Dürauer
astrid.duerauer@boku.ac.at
0043 1 47654-79095

Administrative Assistant of the *Institute of Bioprocess Science and Engineering*

Petra Polak BA
petra.polak@boku.ac.at
0043 1 47654-79084