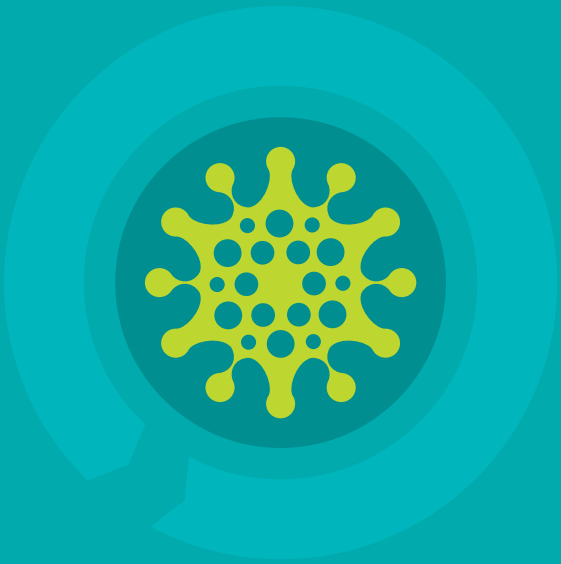
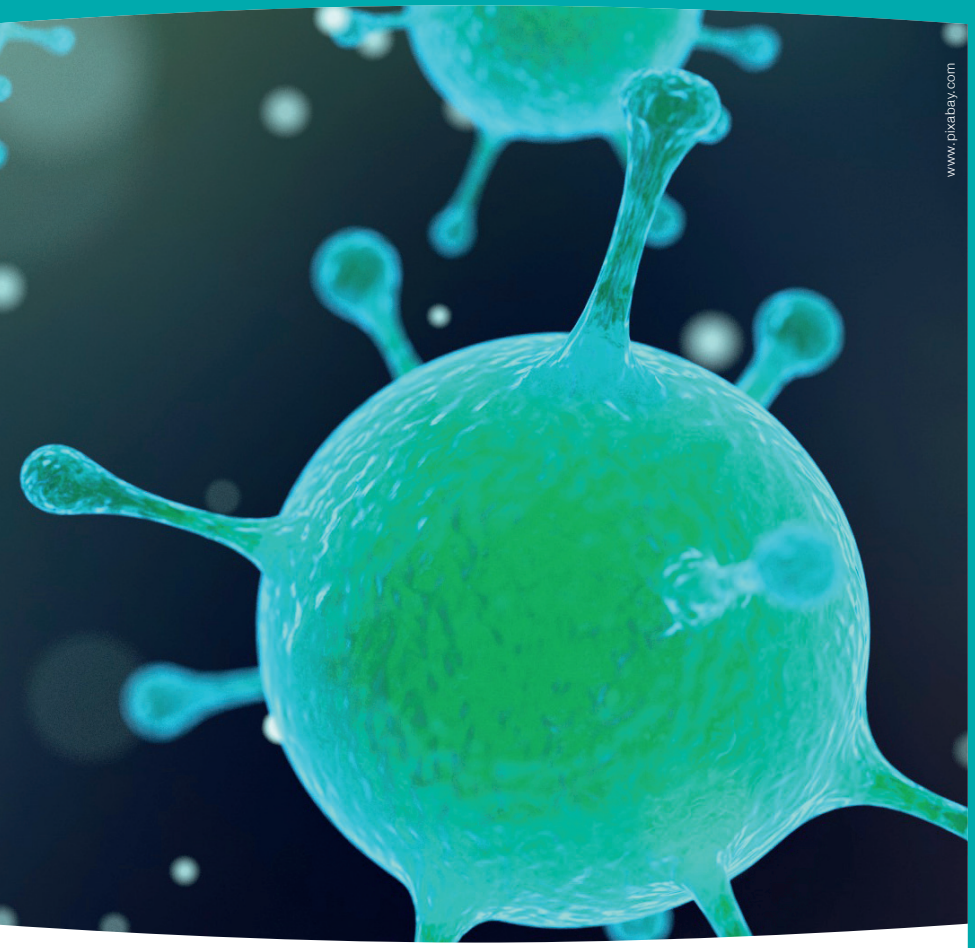
acib austrian centre of industrial biotechnology



Bionanoparticle Process Development



FFG
Forschung wirkt.

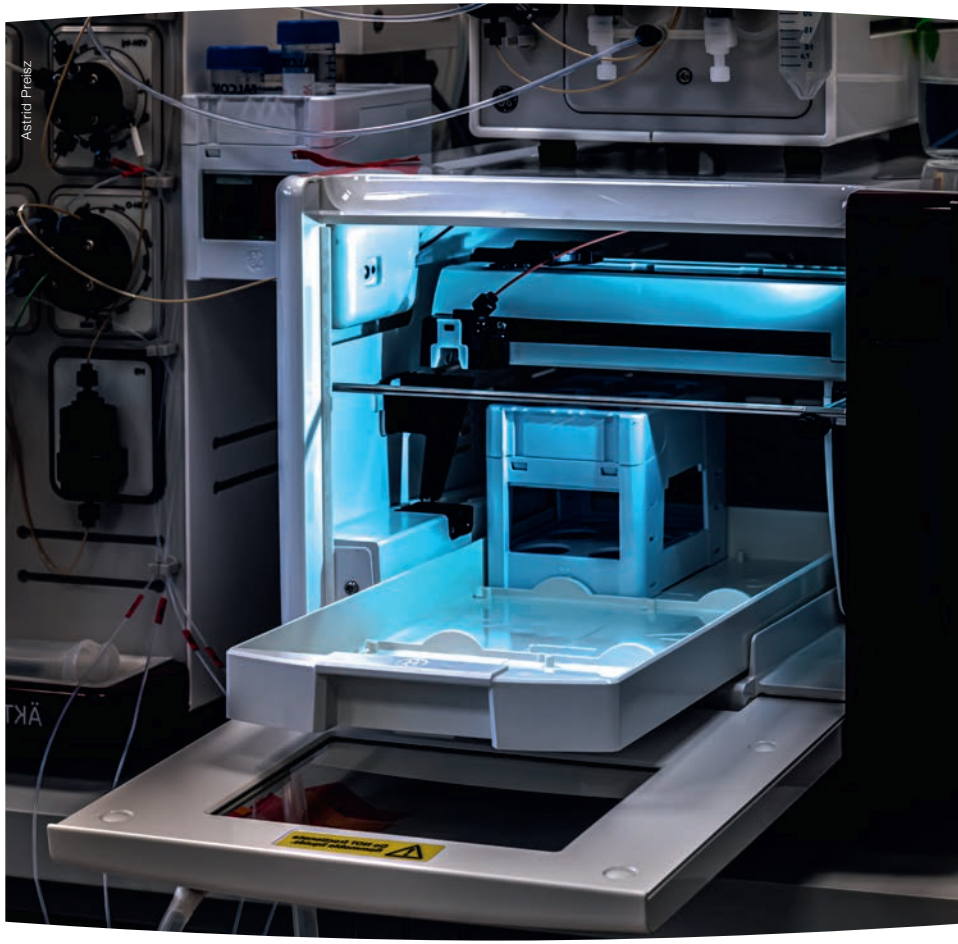
Funding for the infrastructure was received by the Austrian Research Promotion Agency (FFG) through the F&E Infrastrukturförderung“.

Content

Bionanoparticles

THE STATUS QUO

The recent COVID-19 outbreak has shown that the fast development of vaccines is crucial in times of a pandemic. Next to the development of the expression system and production hosts also the design and scale-up of the production process for a vaccine plays an important role in the development chain.
Modern vaccines often consist of bionanoparticles, such as viruses, virus-like particles (VLPs) and extra cellular particles (e.g. exosomes). Apart from its use as vaccines, this product class offers promising candidates for next generation therapeutics, such as vectors for gene-therapy or oncolytic viruses for cancer therapy.
For the use as a modern therapeutic the bionanoparticle composition and biological activity of a certain product must be fully understood. Bottlenecks in rapid process development for such advanced medicines often are the lack of understanding of process mechanisms, the choice of unit operations or the scale-up of production processes.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The Challenges

Upon expression of a therapeutic virus or VLP by a host cell also other kinds of bionanoparticles are budded/shed from the cell contaminating the product. Such contaminants can be apoptotic bodies, exosomes, unknown bionanoparticles, or even other adventitious viruses, which are co-expressed by the host cell. In the case of enveloped bionanoparticles, the contaminants and the product have very similar surface properties and are therefore extremely difficult to distinguish from each other.

The high similarity between the product and impurities makes purification, rapid in-process control and final control of the biopharmaceutical very challenging.

However, the biological properties of biophysically similar bionanoparticles may be completely different; some of them may even cause adverse effects.

Our Solution

At acib, in collaboration with BOKU, we have a proven track record in tackling the problem of bionanoparticle purification (see “Scientific Expertise”). We are also experts in developing fermentation processes and analytical methods, being able to set up integrated processes covering the whole production chain.

Furthermore, acib has established a full set of instruments needed for the development of integrated bioprocesses and analytical methods for the generation of process understanding and for the characterization of bionanoparticle production and purification processes. The infrastructure is located in a non-GMP BSL2 facility at acib/BOKU and can be made available to partners in collaborative projects and also to external users.

Upstream

Parallel bioreactor system

(DASGIP Parallel bioreactor system, Eppendorf)

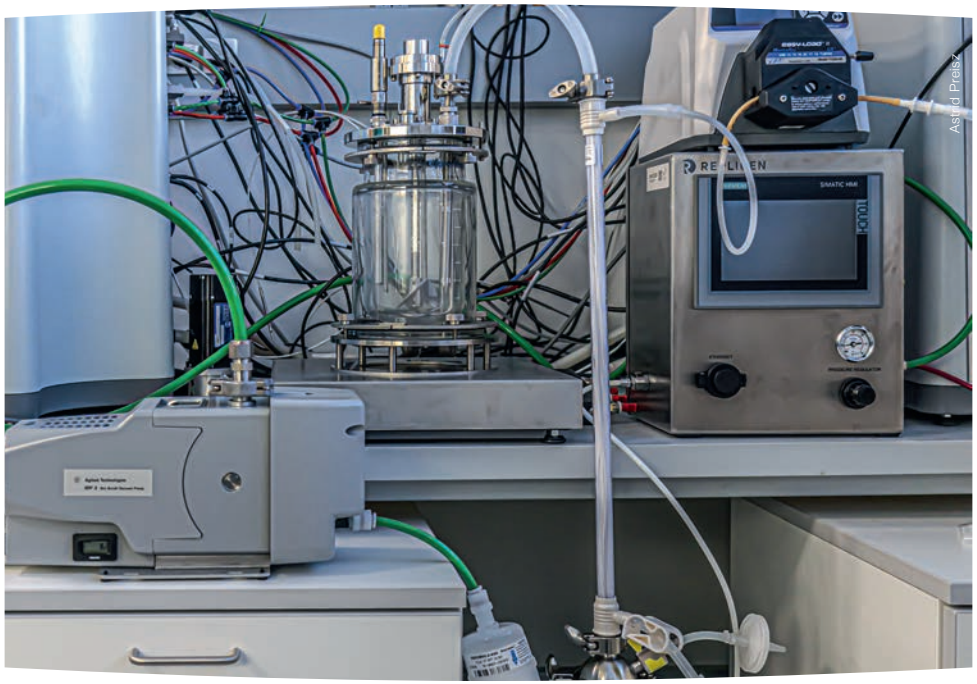
- → Bioreactor system in a scalable benchtop format configured for cell culture and virus applications
- → Parallel processing and precise control of all relevant process parameters
- → Highly suitable for process development and characterisation studies

Upstream

Lab scale bioreactor systems

(BioFlo 320, Eppendorf)

- → Lab-Scale Bioreactor system for cell culture and virus application for scales of up to 10 Liter
- → Single use bioreactor options available
- → Can be used to test for upscale consistency and to provide feedstock supply for downstream development studies



Alternating Tangential Flow system

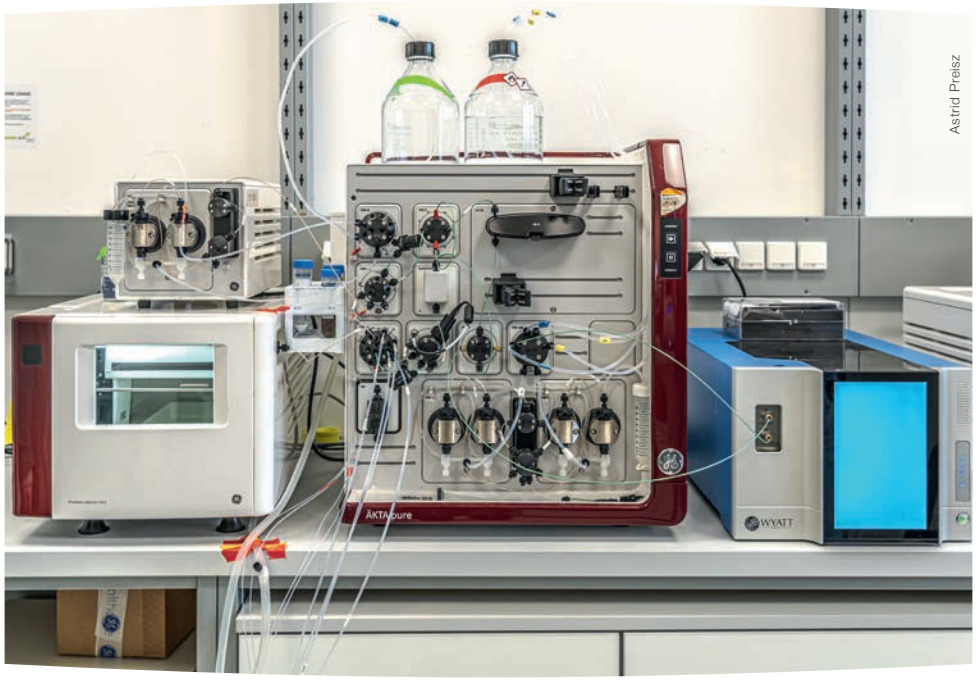
(XCell™ ATF 2, Repligen)

- → Scalable cell retention system delivering high cell concentration and process intensification during cell culture
- → Connects to any bioreactor and can be used in a variety of applications

Downstream

Preparative Chromatography (ÄKTA Pure 150, Cytiva) coupled to **MALS Detector** (Dawn Heleos, Wyatt)

- → On-line detection of particles during chromatographic experiments
- → Accurate tracking of particle elution by light scattering
- → Faster and more efficient DSP development and optimization



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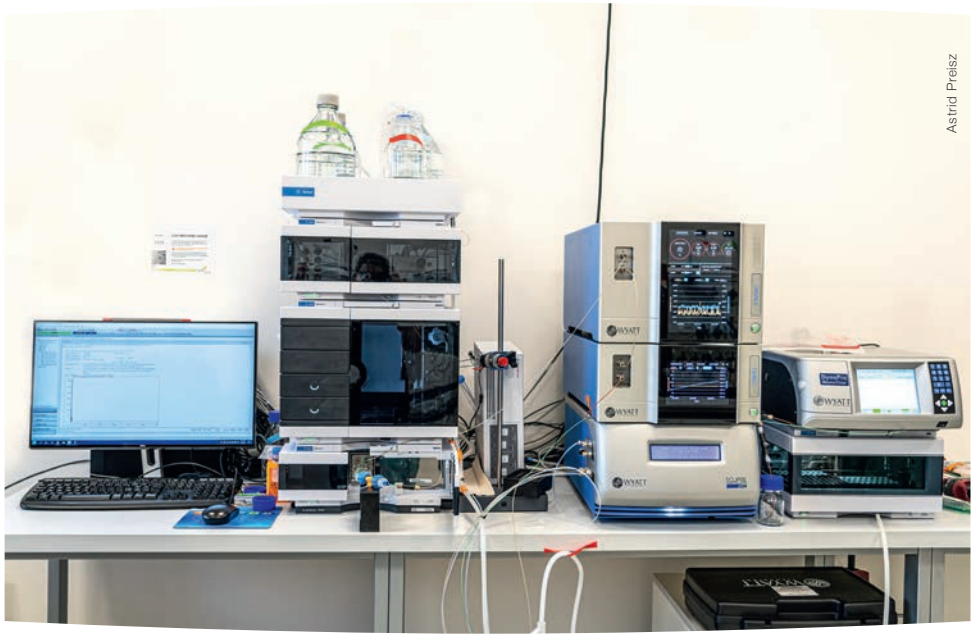
Continuous ultracentrifuge pilot scale (Alfa Wassermann pKII) with **fluid handling system** (Alfa Wassermann AFH)

- → Capture, concentration and primary purification of bionanoparticles directly from crude or clarified harvest material in a single step
- → Fully automated filling and fractionation of ultracentrifuge density gradient processes

Analytics

Asymmetric flow field flow fractionation (A4F) and **analytic HPLC** (Agilent) coupled to **UV-MALS-DLS-RI detectors** (Wyatt)

- A4F: high-resolution separation of bionanoparticles based on hydrodynamic radius in analytical and semipreparative manners
- HPLC: SEC, AEX, HIC and affinity based analytical chromatography
- MALS and DLS: direct determination of particle number, size and shape by multi-angle and dynamic light scattering in the same flow cell
- RI: universal concentration measurement, determination of solvent absolute refractive index



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Analytics

Nanoparticle Tracking Analysis: NanoSight NS300 incl. autosampler (Malvern Panalytics)

- Analysis of the size and concentration of particles in solution by combining the properties of both Brownian motion and light scattering
- Particles are individually tracked, resulting a size distribution of hydrodynamic diameters

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Microflow Cytometer (Apogee)

- Flow Cytometer tuned for extremely small particle applications including extracellular vesicles (EVs), virus and protein aggregates
- Highest sensitivity and resolution from multiple light scattering and fluorescence detectors
- Able to measure biological particles down to 100 nm diameter by light scatter

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What we offer

DEVELOPMENT

→ Upstream processes for bionanoparticles
→ Continuous or non-continuous
→ Mammalian or insect cells

OPTIMIZATION

→ Screening of process parameters

PRODUCTION

→ Bionanoparticle production in lab scale
→ Continuous or non-continuous
→ Mammalian or insect cells

PURIFICATION

→ Downstream processes for bionanoparticles

CHARACTERIZATION

→ Biochemical and biophysical properties
→ Product quality

Scientific Expertise

Recent data can be found in the following references:

Pereira Aguilar, P., Reiter, K., Wetter, V., Steppert, P., Maresch, D., Ling, W.L., Satzer, P., Jungbauer, A. “Capture and purification of Human Immunodeficiency Virus-1 virus-like particles: Convective media vs porous beads” (2020) Journal of Chromatography A.
https://doi.org/10.1016/j.chroma.2020.461378

Pereira Aguilar, P., Schneider, T.A., Wetter, V., Maresch, D., Ling, W.L., Tover, A., Steppert, P., Jungbauer, A. “Polymer-grafted chromatography media for the purification of enveloped virus-like particles, exemplified with HIV-1 gag VLP” (2019) Vaccine.
https://doi.org/10.1016/j.vaccine.2019.07.001

Pereira Aguilar, P., González-Domínguez, I., Schneider, T.A., Gòdia, F., Cervera, L., Jungbauer, A. “At-line multi-angle light scattering detector for faster process development in enveloped virus-like particle purification” (2019) Journal of Separation Science.
https://doi.org/10.1002/jssc.201900441

Strobl, F., Ghorbanpour, S.M., Palmberger, D. et al. „Evaluation of screening platforms for virus-like particle production with the baculovirus expression vector system in insect cells” (2020) Scientific Reports.
https://doi.org/10.1038/s41598-020-57761-w

Strobl, F., Duerkop, M., Palmberger, D. et al. „High shear resistance of insect cells: the basis for substantial improvements in cell culture process design” (2021) Scientific Reports.
https://doi.org/10.1038/s41598-021-88813-4

Reiter, K., Pereira Aguilar, P., Grammelhofer, D., Joseph, J., Steppert, P., Jungbauer, A. „Separation of influenza virus-like particles from baculovirus by polymer-grafted anion exchanger“ (2020) Journal of Separation Science
https://doi.org/10.1002/jssc.201901215

Reiter, K., Aguilar, P.P., Wetter, V., Steppert, P., Tover, A., Jungbauer, A. „Separation of virus-like particles and extracellular vesicles by flow-through and heparin affinity chromatography“ (2019) Journal of Chromatography A
https://doi.org/10.1016/j.chroma.2018.12.035

The Austrian Centre of Industrial Biotechnology

acib uses the concepts of nature to replace traditional industrial methods with new, more economic and ecological technologies. Our goal is bridging the gap between academia and industry. The international non-profit research centre for industrial biotechnology has locations eg. in Vienna, Graz, Innsbruck, Tulln. acib is an international network of 150+ international universities and industry partners.

Owners are the University of Natural Resources and Life Sciences (Vienna), University of Technology (Graz), Universities of Innsbruck and Graz and Joanneum Research.
At acib 150+ scientific employees with up to 25+ years of experience in industrial biotechnology work in more than 100 research projects.

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Save the future, use our technology:

www.acib.at/bionanoparticles

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and watch our **blog** on
www.acib.at/public/acib-blog

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