BOKU Core Facility Biomolecular & Cellular Analysis (BmCA)

BmCA offers state-of-the-art instrumentation and techniques for characterization of biomolecules and/or biomolecular interactions. This also includes X-ray crystallography of proteins. We provide full infrastructure for crystallization and subsequent analysis. Another focus is the analysis and sorting of cells and cellular components by flow cytometry.

General Policy

BmCA offers:

- trained user mode (i.e., direct usage of instrumentation after receiving a training)

or

- full service mode (i.e., experiment performance and experimental report generation by skilled staff)

BmCA staff and the user choose one of these approaches or a combination of both in agreement with each other, depending on the nature of the planned experiment (technical complexity, number of samples etc.), the availability of facility staff, and financial considerations.

Independent from the usage mode and the institution/affiliation of the user, BmCA works on a first-come-first-served basis. Exceptions may be made for urgent experiments (e.g., manuscript resubmissions).

Service fees and prices depend on the legal base of the project for which the internal users of BOKU work. This information (project name, legal base, account number for billing) must be provided by the PI prior to the start of the experiment. According to this information, the PI receives an internal price information. BmCA reserves the right to update prices at any time. External users (i.e., non-BOKU users) receive a customized offer per e-mail after a project discussion and before starting the experiment, which they must confirm in written form (usually per e-mail). Updates of service fees and prices are also valid for ongoing projects.

To start a new project, please contact the facility via bmca@boku.ac.at (see *Communication*) to discuss available options and receive instructions for ordering services. Users are encouraged to have some initial information ready, such as previous attempts, papers showing similar results to what the user wants to achieve, and - last but not least - a clear idea of what one would like to see and what question one would like to answer.

Instrument Usage

List of Instrumentation

- Surface plasmon resonance spectrometer (Biacore T200, GE Healthcare)
- Biolayer interferometer (Octet RED96e, Pall)
- Isothermal titration calorimeter (PEAQ-ITC, Malvern Panalytical)
- Size-exclusion chromatography – light scattering (OMNISEC multi-detector GPC/SEC system, Malvern Panalytical)
- Protein purification (ÄKTA go, Cytiva)
- Differential scanning calorimeter (PEAQ-DSC, Malvern Panalytical)
- Dynamic light scattering – cuvette mode (Zetasizer Nano ZSP with Autotitrator, Malvern Panalytical)
- Flow cytometry – analysis (CytoFLEX S, Beckman Coulter)
- Flow cytometry – cell sorting (SH800S, Sony)

- Flow cytometry – cell sorting (BC MoFlo Astrios EQ, Beckman Coulter)
- Pipetting robot (Mosquito LCP, SPT Labtech)
- Crystal imaging hotel (Rock Imager 1000, Formulatrix)
- Stereomicroscope (SteREO Discovery.V12, Zeiss)
- Protein formulation screen builder (FORMULATOR 10, Formulatrix)

Training

Work in the facility is allowed exclusively after receiving a training by BmCA staff. Users are absolutely not allowed to train other users! Doing so will result in a loss of access to instrumentation by both users. To schedule training, please contact the facility. If a user has not used instrumentation for a period of more than one year, he/she should see the BmCA staff for an update on usage and – if required – retraining. During training, users will also be made familiar with safety regulations. If the training is not successful or if the user does not comply with the rules of the facility, in particular safety rules, BmCA staff can ban individual users temporarily or permanently from using the instruments of the facility.

Sample Quality

Any material tested at BmCA or provided to BmCA staff must be of good quality. We ask the user to evaluate monodispersity/homogeneity of the sample (e.g., via SEC-LS) and have accurately determined the concentration before requesting services. BmCA does not take any responsibility for negative results arising from low quality or errors present in the material given to the facility. For assistance with evaluation of sample quality, please contact BmCA staff. Additional characterization of user-provided samples necessary to perform the requested service may result in additional costs. In case you need support or assistance with regard to sample quality testing, do not hesitate to talk to BmCA staff.

Biosafety and Lab Safety

The use of BmCA services and infrastructure is restricted to material that is classified as safety level S1 (no infectious or hazardous material).

Instrument Booking

Most instrumentation is available to trained users during regular working hours (Mon-Fri, 9-17). For use outside of these hours, please contact the BmCA staff. For BOKU users, flow cytometry instrumentation is possible to be accessed 24/7 after training AND additional approval by BmCA staff. For details, please contact BmCA staff. Instruments and trainings must be booked via our booking software Stratocore (https://ppms.eu/boku/start/). To request a user account, BOKU-internal users have to log in with their BOKU credentials. External users must submit their information (i.e., full name, mail address, phone number, group leader name, billing address) to set up an account in advance via email to bmca@boku.ac.at. All bookings, with the exception of the cytometers, require confirmation by BmCA staff and must be made at least 24 hours before starting the experiment. When the booking is confirmed, the user will receive an e-mail. The use of most instruments (Octet, SPR, ZetaSizer, CytoFLEX, Sony Sorter) is automatically tracked by PPMS (the user must log in to the instruments). For our calorimeters (ITC, DSC) the actual time used is not tracked, but the user must order the number of samples in PPMS. This also applies to the instruments used for protein crystallization. Users must order crystallization packages according to their use. In addition, all users must order consumables they have used (except basic lab equipment e.g., gloves, pipette tips, reaction tubes etc.). Cancellations within 24 hours before training and/or start of instrument time have to be made either personally or by phone, emailing does not guarantee that BmCA staff receives the information in time. Unused booked time slots

must be cancelled. If not cancelled, the booked instrument time will be charged.

No instrument can be booked for more than 7 days in a row. Exceptions can be made for short term external visits or urgent experiments (e.g., for revision of a manuscript). No restrictions for booking instruments apply to BmCA staff for carrying out projects or for maintenance. If instruments become unavailable, BmCA staff will inform all affected users at the earliest possibility. BmCA staff reserves the right to cancel bookings on short notice if instrument maintenance or repair needs to be performed. Preventive maintenance will be scheduled to minimize impact on usage.

It is self-evident that all instrumentation should be used with utmost care, that resources should be used wisely, that users check back with facility staff immediately if anything is unclear or if any problems are experienced with the instrument, and that the instructions laid down in protocols and given by BmCA staff are followed accurately.

User Samples and Reagents

All samples and reagents that are brought to the Core Facility BmCA by users must be clearly labelled with a designation of the compound, the name of the user and the date. For reasons of safety and cleanliness, unlabeled samples will be discarded by BmCA staff without advance warning. During periods of active work, BmCA users are welcome to store their own samples and reagents in designated areas of the facility (please consult BmCA staff in case you want to do so). These areas have to be emptied, however, when the user finishes work. BmCA staff will discard samples and reagents should users not comply with requests to clean up.

In case the full-service mode is used, users will be informed when experiments carried out for them by BmCA staff are completed and whether any material is remaining. Users are kindly asked to pick up any remaining material promptly. Any shipping costs resulting from returning samples to off-campus users must be fully covered by the user.

Waste Management

All (biological) samples (and disposables which they came in contact with) must be discarded in containers labelled with:

Dirty lab ware (bottles, beakers etc.) must be placed on designated trays. Labels must be removed. Methanol-containing waste is generated by using the PEAQ-ITC. It has to be collected in the designated container. Detailed instructions will be given during instrument training.

General Lab Equipment

The following basic lab equipment is available at BmCA:

- Protective equipment (coat, safety glasses, gloves)
- (Multi-channel) pipettes and tips, dispenser pipette
- Plastic reaction tubes (non-sterile)
- Glass bottles and beakers
- Syringes and sterile syringe filters (0.22 µm)
- (Micro) pH-electrodes
- Balances
- Magnetic stirrer (heatable) and vortexer
- Particle-free ultrapure water, mass-spec grade ultrapure water
- Ultrasonic bath (heatable)
- Vacuum pump for filtration of buffers

- Coolable centrifuge (1.5/2 mL reaction tubes, 15/50 mL tubes, plates)
- DeNovix instrument for protein/DNA/RNA concentration determination (absorbance and fluorescence) in microliter or cuvette mode

Users are expected to notify BmCA staff if something is going to run out of stock. Users are encouraged to report any items they miss in the BmCA repertoire.

If items are not returned, are broken or damaged, or are not properly cleaned, the user will be charged for the cost of cleaning or replacement.

Data Transfer and Storage

Raw data generated on BmCA instruments will be stored on the respective instrument control PCs. These PCs are not backed up. Therefore, it is the responsibility of users to transfer their own data immediately after their experiments are finished! ITC and DSC data are transferred by the user with dedicated USB devices (in small boxes beneath the control PC) to one of the two User-PCs. All other control PCs have network connection and data can be transferred directly using drive.boku.ac.at, files.boku.ac.at, BOKU Box, Drop Box etc. If assistance is needed in this respect, please contact BmCA staff!

For the two CytoFLEX control PCs, we delete all data files (.xit and .fcs) older than one year every first Monday in December. Templates (.xitm) and compensation files (.xitc) remain untouched.

USERS ARE ABSOLUTELY NOT ALLOWED TO USE ANY OTHER USB DEVICES THAN THOSE PROVIDED BY BmCA!

The two user PCs are available to BmCA users between Mon-Fri 9-17 without any reservation (on basis of first-come-first-served). All BmCA specific instrument software for data evaluation is available on both PCs (exception: ÄKTA go). Please note that neither the user PCs nor the transfer USBs are intended for data storage. All user data will be deleted from these devices without advance warning.

Communication

Publications

If users present data obtained with support of BmCA (publications or oral/poster presentations), they must acknowledge the facility. Citing the facility in the acknowledgements is the only way we can track the impact of our services. We would appreciate receiving a copy of all publications for which BmCA infrastructure was used. If users need help with preparing a manuscript using data generated via BmCA services (e.g. presentation of data or description of the methodology), please contact BmCA staff. Good scientific practice demands that members of the facility who have contributed intellectually to a publication will be considered as co-authors.

Possible variants:

1. trained user mode (e.g., SPR):

"The SPR equipment was kindly provided by the EQ-BOKU VIBT GmbH and the project was supported by the BOKU Core Facility Biomolecular & Cellular Analysis."

2. full service mode (e.g., SPR by Irene Schaffner):

"We thank Irene Schaffner for conducting surface plasmon resonance experiments. The SPR equipment was kindly provided by the EQ-BOKU VIBT GmbH and the project was supported by the BOKU Core Facility Biomolecular & Cellular Analysis."

3. Core Facility staff member listed as author (e.g., SPR):

"The SPR equipment was kindly provided by the EQ-BOKU VIBT GmbH and the project was supported by

the BOKU Core Facility Biomolecular & Cellular Analysis."

4. Publications resulting from work at the BOKU Core Facility BmCA and the ESRF have to contain an acknowledgment according to the following pattern:

   Within the Material & Methods section, you have to refer to the DOI of your beamline session, i.e.:

"*The data DOI of that session is 10.15151/ESRF-XYZ.*"

   (Your DOI can be found at https://data.esrf.fr/. You can also generate a new DOI if you wish to point to a particular dataset or sets of datasets. You can find more information on this topic at https://www.esrf.fr/ICAT)

   Within the Acknowledgment section please write:

"This project was supported by EQ-BOKU VIBT GmbH and the Protein Crystallography Unit of the BOKU Core Facility Biomolecular & Cellular Analysis. We would like to thank the staff of the ESRF and EMBL Grenoble for assistance and support in using beamline(s) XX, YY, ZZ under proposal number MX2455."

Mailing

To request service or training, please email

bmca@boku.ac.at (general requests)

irene.schaffner@boku.ac.at

elisabeth.laurent@boku.ac.at

georg.schuetz@boku.ac.at

karin.kohlweiss@boku.ac.at

jakob.wallner@boku.ac.at

Feedback and Evaluation

Users are highly encouraged to give feedback about the performance of the facility or suggestions for improvement at any time to the head of BmCA.