CIISB

Czech Infrastructure for Integrative Structural Biology

Equipment and services of CIISB research infrastructure available for **CIISB call** for proposals

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MINISTRY OF EDUCATION, YOUTH AND SPORTS

All Core Facilities accessible through CIISB research infrastructure are part of Instruct - ERIC.

1. Biomolecular Interactions and Crystallography

Core Facility contact: **Josef Houser**, bic@ceitec.cz

Discussion with the Core Facility members is highly advised before planning of your experiments

- General information, methodology selection, SPR, BLI, CD, crystallization: josef.houser@ceitec.cz
- AUC, SEC, MP: monika.kubickova@ceitec.cz
- ITC, DSC, FS, booking system: jitka.holkova@ceitec.cz
- MST, DSF, Cell sorter: eva.fujdiarova@ceitec.cz
- Microarrays: jan.komarek@ceitec.cz
- DLS, SAXS: tomas.klumpler@ceitec.cz
- X-ray diffraction: jaromir.marek@ceitec.cz

Generally, we recommend you to send your requests/comments/questions to the common CF mail (bic@ceitec.cz) to ensure that the responsible person will contact you soon.

Instruments available:

- Analytical ultracentrifuges (**AUC**) **Optima AUC** and **ProteomeLab XL-I** (both Beckman Coulter) – analysis of sample homogeneity and particle size/mass in solution via sedimentation velocity or sedimentation equilibrium techniques. AUC is equipped with absorbance (190 – 800 nm) and interference optics. Two interchangeable rotors: 8-position An-50 Ti and 4-position An-60 Ti. Number of cells for specific purposes (double-sector, six-channel for SE, quartz/sapphire windows, low-volume, etc.)
- Size-exclusion chromatography (**SEC**) with detector array **OmniSEC** (Malvern Panalytical) – analysis of sample homogeneity and particle size/mass using analytical column separation followed by a set of detectors: refractive index (RI), UV-VIS diode array, RALS/LALS light scattering and viscometer. Temperature-controlled autosampler allows to analyze >100 samples in a row. Possibility to collect flow-through fractions.
- Circular dichroism (**CD**) spectroscope **Chirascan V100** (Applied Photophysics) – measurement of circular dichroism in range of 180 – 900 nm. Equipped with Peltier unit for highly accurate temperature control. Broad range of cuvettes from 0.1 to 10 mm path length available.
- Dynamic light scattering (**DLS**) cuvette system **DelsaMAX Core** (Beckman Coulter) – sample homogeneity and hydrodynamic radius of particles in range of 0.2 – 2500 nm. Equipped with static light scattering (**SLS**) detector for determination of molecular mass of particles in

solution. Possibility to adjust temperature in range of 4 – 80 °C and perform temperature-based experiments.

- Dynamic light scattering (**DLS**) plate system **SpectroLight 600** (Xtal Concepts) – high-throughput measurement of sample homogeneity in plates. Ideal for buffer screening, ligand influence on protein stability, etc. with <1 µl sample consumption per condition. Compatible also with measurement in crystallization plates.
- Differential scanning fluorimeter (**DSF**) **Prometheus NT.48** (NanoTemper) – determination of protein thermal unfolding up to 110°C using intrinsic Trp fluorescence. Equipped with back-reflection optics for simultaneous detection of protein aggregation. Up to 48 samples measured in capillaries in parallel.
- Automated differential scanning calorimeter (**DSC**) MicroCal **PEAQ-DSC Automated** (Malvern Panalytical) – direct detection of heat changes during temperature increase or decrease in a range of 4 – 130 °C, at scan rate up to 240°C/hr. Determination of melting temperature, enthalpy of unfolding and change in heat capacity. Automatic sampler ensures high reproducibility, low sample consumption and allows for analysis of >200 samples in one sequence.
- Automated isothermal titration calorimeter (**ITC**) **Auto PEAQ-ITC** (Malvern Panalytical) – direct detection of biomolecular interaction in solution without labelling or immobilization. Autosampler for high reproducibility and small reaction cell for low sample consumption.
- Bio-layer interferometer (**BLI**) **Octet RED96e** (Forte Bio) – high-throughput detection of biomacromolecular binding on surface using up to 8 parallel sensors. Determination of binding affinity, kinetics or active macromolecule concentration in microtiter plates. Various types of sensors for alternative immobilization techniques (streptavidin-biotin, covalent binding, His-tag, Ab-capture, etc.).
- Surface plasmon resonance (**SPR**) system **Biacore S200** (GE Healthcare) – highly sensitive analysis of binding affinity and kinetics. Suitable for broad range of molecule sizes and affinities. Various types of sensors with 4 channels for parallel measurement. Autosampler for up to 384 samples.
- Surface plasmon resonance (**SPR**) **imager** (IPE CAS) – advanced system for specialized SPR measurements on custom-made surfaces. Up to 25 detection spots in standard set-up.
- Microscale thermophoresis (**MST**) **Monolith NT.115** (NanoTemper) – capillary-based analysis of molecular interaction in solution with one fluorescently labelled binding partner. Two excitation wavelengths (blue/red) for broad range of labelling strategies.
- Microscale thermophoresis **(MST) Monolith NT.115 Pico** (NanoTemper) – more sensitive version of Monolith NT.115 for measurement of high affinity (K_D < 1 nM) interactions in solution and/or lower sample consumption. Red excitation laser only.
- Fluorescence spectroscopy **(FS) Fluorolog-QM** (HORIBA) – modular spectrofluorometer designed for advanced fluorescence research with exceptional sensitivity, a wide spectral range (200 – 900 nm), and flexible configuration options. Suitable for steady-state fluorescence experiments including FRET and fluorescence anisotropy. Laser diode for time-resolved fluorescence measurement (at 647 nm). Temperature controlled compartment available.

- Mass photometry **(MP) TwoMP** with **MassFluidix HC (Refeyn)** – measurement of the mass of individual molecules and complexes in solution without requiring labels or extensive sample preparation, non-invasive way to study biomolecular interactions and dynamics. The MassFluidix High concentration microfluidic system - measurement of µM-affinity biomolecular interactions through rapid dilution.
- Imaging system **Odyssey M** (LI-COR) – trans- and epi- illuminator, fluorescence and chemiluminescence scanner with high resolution down to 5 um, compatible with gels, membranes, multi-well plates and microscopy slides
- Crystallization plates storage and inspection **SpectroQ 610** (Xtal Concepts) at 20 °C – for up to 477 SBS-type crystallization plates. Precision temperature control. Automatic drop imaging in visible and UV light. Option to measure dynamic light scattering *in situ* or as dedicated long-term DLS experiment in separate plates.
- Crystallization plates storage and inspection **SpectroQ 210** (Xtal Concepts) at 4 °C – for up to 198 SBS-type crystallization plates. Precision temperature control. Automatic drop imaging in visible and UV light.
- Thermal-control gradient incubator **TG-40** (Centeo) – small-scale temperature optimization of crystallization. 5 different temperatures with up to 8 crystallization experiments each.
- Pipetting robot **Phoenix** (Art Robbins Instruments) – high-throughput setting of crystallization or other SBS-format plates. 96-syrringe head for 0.1 – 100 ul volume transfer. High-speed non-contact dispenser of protein samples for zero cross-contamination.
- Pipetting robot **Mosquito** (TTP Labtech) – nanoliter pipetting (20 – 1200 nl) for advanced crystallization techniques including drop size optimization, hanging-drop set-up, microseeding and co-crystallization experiments. Single-use micropipettes for no cross-contamination. Equipped with LCP module for crystallization of membrane proteins.
- Pipetting robot **Dragonfly** (TTP Labtech) – multidimensional screen preparation. Non-contact dispensing of up to 5 different solutions in a single run. SBS-type plates compatible.
- Pipetting robot **epMotion 5075l** (Eppendorf) – versatile pipetting robot for SBS-type plates, tubes and microtubes. Liquid transfer, aliquoting, dilution series, etc.
- Ultra-low volume dispensing system **sciFLEXARRAYER S1** (Scienion) – non-contact piezo dispensing of 50 – 800 pl droplets onto surfaces. Suitable especially for sensor chip arrays and biosensor modifications.
- Cell sorter **CytoFLEX SRT** (Beckman Coulter) – advanced instrument for cell counting and sorting capable of complex sort logic, including 4-way sorting.
- Diffraction system **XtaLab Synergy** (Rigaku) – robotized macromolecular diffractometer (**PXD**) with sample changer ACTOR optimized for work at Cu-Kα wavelength
- Small-angle X-ray scattering **(SAXS)** camera **BioSAXS-2000** (Rigaku) – equipped with automatic sample changer for SAXS (small angle X-ray scattering) experiments with solutions of biological macromolecules and with manually filled sample holders for SAXS experiments with nanostructures

Services provided:

- Calorimetric measurement of molecule-molecule interactions using automated ITC (standard titration, singe-injection method, competitive titration)
- Calorimetric measurement of macromolecular stability using automated DSC (melting point, enthalpy of denaturation, reversibility of denaturation)
- Analysis of macromolecular sample homogeneity, oligomeric state and/or interaction by analytical ultracentrifugation
- Analysis of biomacromolecular sample homogeneity, size and/or oligomeric state by analytical ultracentrifugation or by size-exclusion chromatography with light scattering detector
- Complete protein biophysical characterization of user samples – determination of homogeneity (tendency for aggregation), temperature stability and identity (MS-determined intact mass in collaboration with Proteomics Core Facility of CEITEC)
- SAXS experiments with solutions of biological macromolecules or with non-biological samples
- All other available biophysical techniques may be offered as service upon agreement with CF staff as well
- Preparation of customized protein arrays on glass slides
- Set-up of crystallization screens (drop volume 200 nl) using 30 different commercial kits (including kits for protein complexes, membrane proteins and nucleic acids) – sitting drop, hanging drop or under oil batch method
- Automatic storage of the screening plates (4°C or 20°C) with visible light imaging. Optional UV imaging to distinguish protein from salt
- Setting-up multidimensional gradients plates for optimization of crystallization and other purposes
- Advanced crystallization techniques – optimization screens, additive screens, seeding, crystallization in capillaries, LCP crystallization, crystallization by dialysis, tools for manual crystallization set-up, estimating of precipitation diagram, heavy atom compounds derivatization for phasing (over 70 different compounds available)
- Test of a diffraction quality of protein crystals, derivatives, cryoprotectants, etc. prior data collection
- Collection of diffraction data with crystals of biological macromolecules
- Procession of diffraction data and solving of structures of biological macromolecules by MR, MIR, SAD, MRSAD and/or MAD methods
- Training of the users for independent work on the instruments and in data analysis

2. Nanobiotechnology Core Facility

Core Facility contact: **Jan Přibyl**, jan.pribyl@ceitec.muni.cz

Group email: nanobiocf@ceitec.muni.cz (will reach all members of CF)

Nanobiotechnology CF helps researchers (structural biologists, biochemists, and chemists) better understand the complex cellular processes, obtain nano-meter scale images of the single biomolecules, and characterize nano-objects and their complexes with biomolecules. The instruments available within the CF allows to monitor biological samples in their native state and in their physiological environments, thus allowing a better understanding of the behaviour of complex biological systems. However, not only the structural properties can be studies, also the biomechanics and chemical composition of single biomolecules, nano-objects, living cells and tissues can be studied by either individual use or in-situ combination of instruments available in our labs (AFM probe microscopy, optical microscopy, Raman microscopy, multielectrode array). Biosensor platforms support the obtained data by high-throughput analysis of biomolecular interaction.

Services provided:

The imaging and mechanical characterization of biomolecules, their complexes, living cells and other biological structures (tissue sections) and objects is realized in aqueous (buffered) solutions or in the dry state. Different AFM probes, including the spherical indentors, are available. The carrier materials for sample deposition range from ultra-flat mica slides suitable for atomic resolution to highly oriented graphite, gold, silicon, glass, and polymers as polystyrene petri dishes.

- AFM imaging and mechanical characterization of nano-objects (nanoparticles, nanorods, etc.), biomolecules and their complexes in dry or under nearly physiological conditions (buffered environment, elevated temperatures).
- Structural and mechanical characterization of individual living cells, including adhesive studies by cell-scratching and FluidFM aspiration.
- Preparation of samples for AFM – optimization of immobilization process.
- AFM combined with other techniques: electrochemistry, optical and confocal fluorescence microscopy.
- Protein folding-unfolding studies by automated AFM technology (SMFS, single molecule force spectroscopy).
- Multielectrode Array - characterization of field potential of electrically active cells (cardiac cells, neurons). nanolithography, nanomechanical manipulations, ink-jet based deposition.
- AFM measurements, statistical analysis and data filtration, mechanical, electric (KPFM) and magnetic (MFM) properties of samples.
- Production, bioconjugation and deposition of nanoparticles (gold, magnetic, fluorescent QDs and other core/shell structures, upconverting, ...) .

The Core Facility Staff provides the users design and planning of experiments, operation of instruments, and help with data analysis, including access to the evaluation software.

List of services and main instrumentation:

Cells – imaging or mechanical properties

Living cells in either standard plastic Petri or confocal dish. Force Mapping of cell cultures, mechanical characterization of cardiomyocytes. Possible combination with optical microscopy (bright field, fluorescence) – independent/overlay imaging with AFM. All operations are under semi-physiological conditions, i.e., in buffered solutions (culturing media), CO2 atmosphere, and elevated temperature (RT to 60 oC).

- **Instruments available:**
  - AFM JPK NanoWizard® 3 combined with Olympus IX-81/FV1200
  - AFM JPK NanoWizard® 4XP combined with Leica DMi8
  - Bruker Hysitron BioSoft Indentor
- BioAFM for large samples combined with BF/fluoresce/confocal microscopes for correlative microscopic approaches. Maximal scanning area NW3 100x100x15 µm, NW4XP 200x200x200 µm allows continuous operations on large samples.
- Hysitron Biosoft Indentor can be used for elastic and viscoelastic characterization of soft samples such as tissues or hydrogels. Combination of force sensitivity and displacement range (up to 100 µm) allows accurate and repeatable indentation experiments.
- **Modes:** Contact mode, Tapping Mode, Quantitative Imaging – hybrid mode for combined mechanical and topographical characterization (QI), Force Mapping – full indentation mode (PF-QNM), Indenation, Load-relaxation test.
- **Samples and analysis:** Structural and mechanical analysis of single cells (cell cultures), cell clusters (nanoparticles, nanorods, nanosheets, etc.), tissue slices, whole organisms (small plants) and hydrogels.
- **Correlative microscopic approaches** – the combination of experiments with BF/fluorescence/polarization microscopy.
- **FluidFM** extension can be used as nano/micropipette with precise force control for cell adhesivity studies.
- **ForceRobot® 300,** fully automated system for collecting force spectroscopy for single-molecule force spectroscopy studies.

Biomolecules or nano-objects - imaging

AFM imaging of biomolecules (proteins, DNA, macromolecules, and biopolymers), nano-objects (nanoparticles, nanotubes, nanorods, etc.) and their complexes. Immobilization on mica (muscovite), other materials (HOPG, silicon, metal electrodes) can also be used. All operations can be under semi-physiological conditions, i.e., in buffered solutions and elevated temperature.

- **Instruments available:**
  - AFM JPK NanoWizard® 3 combined with Olympus IX-81/FV1200
  - AFM JPK NanoWizard® 4XP combined with Leica DMi8
  - Bruker Dimension FastScan, Bruker Dimension Icon
- Bruker Multimode High speed and resolution AFM with ScanAsyst semi-automatic control, imaging in liquid.
- **Modes:** Contact mode, Tapping Mode, Force Volume, Peak Force Quantitative NanoMechanics (PF-QNM).
- **Samples and analysis:** Structural and mechanical analysis of single biomolecules (DNA, RNA, proteins, oligonucleotides, and their complexes), nano-objects (nanoparticles, nanorods, nanosheets, etc.) and their complexes with biomolecules, nano- and micro-plastic particles, aggregates, viruses, spores, microorganisms.

Raman microscopy

- Chemical mapping of different samples, including large variety of biosamples – nanomaterials, cells, tissue samples (bones, Alzheimer fibres, etc.), plant composition, etc. Microplastic and nanoplastic particle analysis. **Instruments available:** Renishaw Raman Microscope.
- The inVia microscope comprises a research-grade microscope coupled to a high-performance Raman spectrometer. Performance—high signal throughput, combined with high spectral resolution and stability—giving reliable results, for even the most challenging measurements. For more detailed information, download the inVia microscope brochure available on CF website.

Samples and analysis: Chemical mapping of different samples, including large variety of biosamples – nanomaterials, cells, tissue samples (bones, Alzheimer fibres, etc.), plant composition, etc. Microplastic and nanoplastic particle analysis.

Measurement of luminescence

Scanning of upconversion luminescence from surfaces, including microtiter plates, nitrocellulose membranes, or gels. Excitation at 980 nm, measurement of emission at 540 or 810 nm. The additional possibility of multiplexing with common fluorescence dyes (FITC, TRITC) and absorbance measurements. Evaluation of average intensities in microtiter plate wells or creation of 2D map of upconversion signal.

- **Instruments available:** Upconversion scanner Labrox scanner

Multielectrode array recording of cellular potential

A microelectrode array is a grid of tightly spaced microscopic electrodes embedded in the bottom of each well in a multi-well MEA plate. Cells, such as cardiomyocytes or neurons, which are electrically active, can be cultured over the electrodes creating a cohesive network. The functional behavior or electrical activity of this network can be recorded. These action potentials are recorded extracellularly and are known as field potentials. Easy combination with JPK NW3 and Olympus IX-81 microscopes for combined electrophysiological-biomechanical and optical measurements.

- **Instruments available:** Multichannel MEA2100-Mini + AFM JPK NanoWizard® 3
- **Samples and analysis:** Record spontaneous activity from hiPSC-derived neuronal cells upon differentiation and maturity, cell cultures for disease modeling such as epilepsy, drug screening or neurotoxicology studies or study functional maturity of cardiomyoctyes.
- Mechanoelectrical coupling/decouling studies by combining the MEA with AFM and optical microscopes.

3. Josef Dadok National NMR Centre

Core Facility contact: **Pavel Kadeřávek**, pavel.kaderavek@ceitec.muni.cz

Core Facility of High Field NMR Spectroscopy provides access to NMR spectrometers in the range of proton frequencies from 500 MHz to 950 MHz. The equipment is suited mainly to the studies of structure, dynamics, and interactions of biomolecules, i.e., proteins, nucleic acids and carbohydrates. However, the instrumentation is flexible enough to cover also various research needs in material science, organic and inorganic chemistry, biochemistry, biology, and biophysics.

Instruments available:

- NMR Spectrometer Bruker AVANCE NEO 500 MHz – available with a 5 mm nitrogen-cooled multinuclear cryoprobe (Prodigy), room temperature 5 mm dual broad-band probe (1H, 15N - 19F), triple-resonance (1H, 13C, 15N) 5 mm probe, 10 mm dual (1H, 13C) probe, and 4.0 mm solid-state dual CP/MAS probe.
- NMR Spectrometer Bruker AVANCE NEO 600 MHz equipped with a quadruple-resonance (1H/19F, 13C, 15N, 31P) cryoprobe with -40 to 150ºC temperature range.
- NMR Spectrometer Bruker AVANCE NEO 700 MHz for biomolecular applications, equipped with a triple-resonance (1H/19F, 13C, 15N) cryoprobe with -40 to 150ºC temperature range and a triple-resonance (1H, 13C, 15N) cryoprobe optimized for detection of 13C, -40 to 80ºC temperature range.
- NMR Spectrometer Bruker AVANCE NEO 700 MHz for multinuclear applications, equipped with a 5 mm dual broad-band probe, 5 mm dual inverse broad-band probe, 1.7 mm triple resonance (1H, 13C, 15N) probe, 5 mm dual broad-band probe for diffusion measurements, 3.2 mm high-resolution solid-state triple-resonance (1H, 13C, 15N) MAS probe, and 4.0 mm solid-state dual CP/MAS probe.
- NMR Spectrometer Bruker AVANCE NEO 850 MHz equipped with a triple-resonance (1H/19F, 13C, 15N) cryoprobe with 0 to 135ºC temperature range and a triple-resonance (1H, 13C, 15N) cryoprobe optimized for detection of 13C, -40 to 150ºC temperature range.
- NMR Spectrometer Bruker AVANCE NEO 950 MHz equipped with a triple-resonance (1H/19F, 13C, 15N) cryoprobe, -40 to 150ºC temperature range.
- X-Band (9,75 GHz) EPR spectrometer Bruker EMX nano.
- Bioreactor system for NMR studies of proteins/nucleic acids in living mammalian cells

Services provided:

- Measuring of NMR spectra at magnetic fields from 11,75 T to 22,32 T (corresponding to proton frequencies from 500 MHz to 950 MHz) for compounds identification and quality control.

- Consultations concerning the choice and setup of multidimensional NMR experiments, data processing and spectra evaluation according to the users' needs.
- Structure analysis of organic compounds by NMR spectroscopy.
- Studies of structure and dynamics of proteins and nucleic acids by NMR including measurement and processing of multidimensional spectra of proteins with up to 5 dimensions using non-linear sampling.
    - Mapping of minor conformations (invisible states) by relaxation dispersion.
    - Studies of interactions using STD (Saturation Transfer Difference) spectroscopy.
    - *In vivo* conformational studies of biomolecules in cell.
    - Investigation of material structure by solid state NMR spectroscopy of ^{13}C, ^{31}P, ^{29}Si, and ^{27}Al.

4. Cryo-electron Microscopy and Tomography

Core facility contact: **Jiří Nováček**, jiri.novacek@ceitec.muni.cz

The Cryo-electron microscopy core facility CEITEC Masaryk University (CEMCOF) provides access to the state-of-the-art electron microscopy instrumentation for cryo/RT-EM research in structural and cellular biology using single particles analysis, cryo-electron tomography, and (cryo-)volume EM. As a part of our mission, we support electron microscopy workflows starting from the sample preparation and optimization for the cryo-EM towards advanced sample preparation techniques such as focused ion beam micromachining of cellular lamellae or cryo-volume EM data acquisition. In addition, the facility gradually adopts emerging techniques in cryo-EM such as electron diffraction tomography (micro-ED) and has capacities for cryo-EM data analysis. The facility can handle BSL1 and BSL2 specimens, respectively, and is available to its users for both service and collaborative projects.

Instruments available:

- Titan Krios – 300 kV high-end transmission electron microscope aligned for fringe-free imaging (FFI) and equipped with Volta phase plate (VPP), energy filter, and Ametek K3 direct electron camera (Bioquantum K3). The microscope serves for high-end single particle, cryo-ET, and micro-ED data collection.
- Talos Arctica – 200 kV high-end transmission electron microscope aligned for FFI and equipped Selectris energy filter, Falcon 4i direct electron detector, and a scintilation detector (Ceta-D) for imaging. The microscope serves for acquisition of single particle analysis and cryo-ET data, and high-throughput screening of cryo-EM samples.
- Glacios 2 – 200 kV high-end (scanning-)transmission electron microscope equipped with Falcon 4i direct electron detection camera, scintillation camera (Ceta), and a set of bright-field, dark-field, and high-angle annular dark-field detectors (Panther STEM) for imaging. The microscope serves for single particle cryo-EM data acquisition, acquisition of cryo-STEM tomography data, and high-throughput screening of cryo-EM specimens.
- Talos F200C – 200kV (scanning-)transmission electron microscope equipped with Falcon 3EC direct electron detector, Ceta-D camera, and Quadro direct electron detection camera. In addition, the microscope is equipped with HAADF detector for STEM imaging and Bruker X-flash detector for EDX mapping. Talos F200C is multi-purpose microscope for life-science and chemistry applications.
- Arctis - tripple beam (FM/FIB/SEM) microscope equipped with non-immersion SEM column, ETD, T1, T2 detectors, Autoloader, and plasma focused ion beam (three gases – Xe,Ar,O) source for specimen ablation. The microscope is used for high-throughput (targetted) cellular lamella preparation from samples on TEM grids of prepared by „Waffle“ method.

- Helios V Hydra – tripple beam (FM/FIB/SEM) microscope with immersion SEM column for high-resolution SEM imaging. The data can be captured using ETD, ICD, TLD, or ICD detectors. The instrument is equipped with the plasma focused ion beam (four gases – Xe,Ar,O,N) source for efficient milling of biological specimens, cryo-stage, cryo-liftout, and integrated wide-field flourescence microscope. The microscope is used for cryo-FIB lamella preparation and volume EM data acquisition at room-temperature and cryo-conditions.
- Helios V HydraBio – dual beam (FIB/SEM) microscope with immersion SEM column for high-resolution SEM imaging. The data can be captured using ETD, ICD, TLD, or ICD detectors. The instrument is equipped with the plasma focused ion beam (four gases – Xe,Ar,O,N) source for efficient milling of biological specimens, and cryo-stage. The microscope is used for cryo-FIB lamella preparation and volume EM data acquisition at room-temperature and cryo-conditions.
- Versa3D – small dual beam (FIB/SEM) equipped with cryo-stage and cryo-FIB/SEM preparation system (Quorum Technologies). The microscope primarily serves for preparation of cellular lamellae by cryo-FIB for cryo-electron tomography.
- Leica CryoCLEM - wide-field flourescence microscope with cryo-stage and 50x cryo-objective (NA 0.9) for correlative light-electron microscopy research on vitrified biological specimens.
- Chameleon – vitrification device for automated spray&freeze sample vitrification by plunge freezing. The instrument contains integrated glow discharge unit and piezoelectric dispensor coupled with syringe pump for automated sample deposition to TEM grids which can achieve spray-to-plunge times of 100 ms – 2.5 s. The device is used for automated sample preparation for single particle cryo-EM for and for time-resolved cryo-EM experiments.
- Instrumentation for sample preparation – vitrification robots (ThermoScientific Vitrobot Mark IV, Leica EM GP2) are available for plunge freezing of specimens for cryo-EM. A high-pressure freezer (Leica EM ICE), freeze-substitution unit (Leica EM AFS2), and cryo-ultramicrotome (Leica EM UC7) are available for preparation of cellular or tissue samples for electron microscopy under room-temperature or cryo-conditions.

Services provided:

Our mission is to provide access to the state-of-the-art instrumentation for electron cryo-microscopy and support our users in sample preparation, data acquisition, and data processing. The facility provides trainings to its users for independent operation of all the available instrumentation and trainings in sample preparation. Data analysis is available through collaboration. The services provided by the facility comprise:

- TEM imaging
- SEM imaging
- cryo-flourescence imaging
- sample vitrification (plunge freezing & high-pressure freezing)
- freeze-substitution
- resin embedding

- (cryo-)volume EM
- initial structural screening[1]
- initial cryo-EM screening[2]
- (targeted) cryo-FIB lamella preparation
- single particle analysis data collection
- cryo-electron tomography data collection
- electron diffraction tomography
- data analysis[3]

[1] Sample preparation and imaging by negative staining EM to support sample suitability for structural characterization by electron microscopy.
[2] Optimization of sample vitrification conditions and sample imaging to provide sufficient data to prove sample quality for high-end data collection.
[3] Available in limited number, prolonged service duration possible.

5. Proteomics Core Facility

Core Facility contact: **Zbyněk Zdráhal**, zbynek.zdrahal@ceitec.muni.cz

The core facility provides the academic and other entities with access to advanced MS proteomic technologies based on shared resources and the know-how of highly trained staff. The services cover all the steps of mass spectrometry-based proteomic analyses from protein isolation, protein and peptide separation (liquid chromatography, electromigration methods), to protein characterisation by mass spectrometry, data processing with statistical evaluation, preparation of final reports and optionally basic bioinformatic analysis.

It operates predominantly in a full service mode. We provide also consultancy in the field of our expertise (e.g. selection of the optimal proteomic application, designing proteomic experiments).

Instruments available:

- MALDI-MS/MS – Ultraflextreme mass spectrometer
- LC-MS/MS I – Ultimate 3000 RSLCnano + Orbitrap Exploris 480 mass spectrometer (incl. FAIMS)
- LC-MS/MS II – Ultimate 3000 RSLCnano + Orbitrap Fusion Lumos Tribrid mass spectrometer
- LC-MS/MS III – nanoElute + timsTOF Pro mass spectrometer
- LC-MS/MS IV – Evosep One + timsTOF HT mass spectrometer
- LC-MS/MS V – nanoElute 2 + timsTOF Ultra 2 mass spectrometer
- LC-MS/MS VI – Ultimate 3000 RSLCnano + Impact II Qq-Time-Of-Flight mass spectrometer

Services provided:

- analysis of intact proteins (MALDI-MS profilling)
- protein identification (incl. protein complexes characterization, de novo sequencing)
- characterization of protein modifications
- absolute and relative protein quantification

You can contact us about availability of other proteomic applications.

6. Biophysical techniques

Core Facility contact: **Táňa Černovec**, tana.cernovec@ibt.cas.cz
Venla Mustonen, venlasarasofia.mustonen@ibt.cas.cz

Instruments available:

- Monolith microscale thermophoresis (MST) NT.115 instrument (Nano Temper)
- Monolith microscale label free thermophoresis NT.LabelFree instrument (Nano Temper)
- Surface Plasmon Resonance (SPR) system ProteOn XPR36 (BioRad)
- Isothermal titration calorimeters MicroCal iTC200 and MicroCal PEAQ-ITC (Malvern Panalytical)
- Differential scanning calorimeter MicroCal VP-Capillary DSC (Malvern Panalytical)
- Circular dichroism (CD) spectrometer Chirascan Plus (Applied Photophysics)
- UV/Vis Spectrometer Specord 50 Plus (Analytik Jena)
- Spectrofluorimeter FLS1000 (Edinburgh Instruments) with a SuperK EXTREME, a supercontinuum white light laser (NKT Photonics)
- Fourier-transformed Infrared (FTIR) spectrometer Vertex 70v (Bruker)
- Differential scanning fluorescence (DSF) assay Prometheus NT.48 (Nano Temper)
- Zetasizer Nano ZS90 (Malvern Panalytical) dynamic light scattering (DLS) technique
- Zetasizer Ultra (Malvern Panalytical) multi-angle dynamic light scattering (MADLS) technique
- Mass Photometer Two MP (Refeyn)
- Microplate multimode reader Spark (Tecan)
- Bio-Layer Interferometry Octet R8 (Sartorius)
- HeliX switchSENSE dynamic biocensors technology (Bruker)

Services provided:

All services at CMS (Centre of Molecular Structure of BIOCEV) are available both to unexperienced users and to experienced users (that do not require the assistance of the scientist in charge)

- Applied Photophysics Chirascan Plus CD spectrometer – measurement of circular dichroism spectra and absorbance as function of temperature, pH and concentration to determine the secondary structure of proteins and peptides, conformation of RNA and DNA, as well as to detect conformational changes;
- Analytic Jena Specord 50 Plus – molecular absorption spectroscopy with ultraviolet and visible radiation in the spectral range from 190 to 1100 nm;
- BioRad ProteOn XPR36 – label-free quantitative analysis of biomolecular interactions by the technique of surface plasmon resonance (SPR);
- Malvern Microcal iTC200 and MicroCal PEAQ-ITC– label-free solution studies of biomolecular interactions;

- Malvern Microcal VP-DSC – direct measurement of intramolecular stability of biological macromolecules, as well as the intermolecular stability of biologically-relevant complexes such as oligomeric proteins, nucleic acid duplexes, and micellar systems (lipid and detergent micelles);
- Nano Temper Monolith NT.150 – to study biomolecular interactions. The device allows to characterize protein-protein and protein-ligand (small molecule, DNA, RNA, peptides, sugars, lipids) interactions that can be measured under close to native conditions based on thermophoretic effect. Protein labeling is required with this device;
- Nano Temper NT.LabelFree – characterization of protein-ligand interactions based on thermophoretic effect, using the intrinsic tryptophan fluorescence. No sample modification is required with this device;
- Nano Temper Prometheus NT.48 – measurement of protein stability using tyrosine and tryptophan fluorescence;
- Malvern Zetasizer Nano ZS90 – measurement of molecular size using Dynamic Light Scattering (DLS), zeta potential and molecular weight using Static Light Scattering.
- Malvern Zetasizer Ultra – fast and accurate particle measurements by Multi-Angle Dynamic Light Scattering (MADLS) with Non-Invasive-Back-Scatter (NIBS);
- FLS1000 Photoluminescence Spectrometer – for measuring spectra from the ultraviolet to the mid-infrared spectral range, and lifetimes spanning from picoseconds to seconds;
- Vertex 70v Fourier-transform Infrared (FTIR) Spectrometer provides the unique possibility to acquire a complete far and mid IR spectrum from 6000 cm-1 to 50 cm-1 in a single step measurement;
- Mass Photometer Two MP - the measuring molecular mass of molecules in solution;
- Microplate multimode reader Spark for the absorbance, fluorescence, fluorescence polarization, and luminescence measurements;
- Octet R8 – for the label-free analysis of biomolecular interactions by the technique of Bio-Layer Interferometry;
- HeliX device is using switchSENSE dynamic biocensors technology for the measurement of binding kinetics (association rate k_{on}, dissociation rate k_{off}), affinity, ternary complex formation / proximity induced binding, conformational changes and relative changes in protein size, nucleic acid enzyme binding and activity.

Details of instruments and methods:

Circular Dichroism (CD) spectropolarimeter

The Chirascan Plus CD spectropolarimeter with avalanche photodiode detector - provides fast scanning and high sensitivity. This instrument can simultaneously measure accurate CD, absorbance and fluorescence data.

- Detection range: 170-1150 nm;

- Peltier temperature control.

Circular dichroism can be used for:

- Determination of protein folding;
- Characterization of protein secondary structure and DNA conformation;
- Detection of the changes in protein structure upon mutagenesis;
- Studying of conformational stability of proteins and DNA (pH stability, denaturant stability, temperature, buffers addition of stabilizers).

Data processing:

- The CDNN software package is available for detailed model-based analysis and predicting secondary structure using CD data;
- Software Global Analysis of multiwavelength kinetic data is available to fit multi-dimensional experimental data to one of a number of specified models.

Sample requirements:

- Measurement of CD spectrum for the determination of secondary structure of protein requires 160 µl of 0.1 – 0.2 mg/ml protein solution;
- Measurement of CD spectrum for the determination of DNA conformation requires 160 µl of 20 µM of solution or 1400 µl of 2 µM solution;
- Not optimal for CD solutions, containing DTT, imidazole, glycerol, DMSO, high concentrations of salts.

UV/Vis Spectrophotometry

The AnalyticJena SPECORD 50 PLUS device is a UV/Vis double-beam spectrophotometer with split-beam technology that combines high energy throughput with good stability.

- 190-1100 nm;
- 50-1500 µl of sample;
- Scanning, dual beam
- Temperature control with Peltier element, scan-range 5-95ºC.

The spectrometer can be used for:

- Proteins and DNA thermostability measurements;
- With or without stirrer can be used for enzyme kinetics.

Surface plasmon resonance (SPR)

The ProteOnTM XPR36 protein interaction array system enables label-free quantitative analysis of biomolecular interactions in real time using SPR technology. The ProteOn system allows to screen analytes simultaneously against 36 different targets of interest, enabling rapid comparison among large numbers of interactions.

SPR can be used for:

- Quantification of binding affinity and kinetics;
- Determination of binding specificity and the number of binding sites;
- Characterization of membranes, lipids, nucleic acids and micellar systems.

Sample requirements:

- Concentration of ligand depends on the level of immobilization desired, generally 10–200 µg/ml. For kinetic analysis the best results are obtained by using a 100-fold range of analyte concentrations, 0.1–10xKd;
- Immobilization of one interacting partner is essential. The service can provid with a sensor chip, or with the user bringing own chip;
- The ProteOn acetate buffer (at pH 4.0, 4.5, 5.0, or 5.5) is recommended as immobilization buffer;
- The recommended running buffer for most applications is the ProteOn phosphate buffered saline, pH 7.4 (10 mM sodium phosphate and 150 mM sodium chloride with 0.005% Tween 20).

Isothermal Titration Calorimetry (iTC)

The Malvern iTC200 instrument is used for the characterization of biomolecular interactions of small molecules, proteins, antibodies, nucleic acids, lipids etc.

The iTC200 device can be used for:

- Direct measurement of submilimolar to nanomolar binding constants (103 - 109 M-1);
- Thermodynamic characterization of the molecular interaction in a single experiment (stoichiometry, Kd, ΔH and ΔS values);
- Calorimetric measurement over a range of biologically relevant conditions (temperature, salt, pH, etc.).

Sample requirements:

- The buffer solution, containing both the macromolecule and the ligand of interest, should be the same.

- The volume of the sample placed in the cell must be at least 300 µl. Preferably, the solutions of macromolecules should be dialysed against the buffer solution used for the ITC measurement;
- The ligand solution (the sample placed in the injection syringe) must have a volume at least 70.0 µl. Normally the ligand concentration should be 10 times as high as the concentration of macromolecule;
- In the case of high affinity interactions, the minimum concentration of macromolecule (that causes measurable heat effects) is 10 µM. For low affinity interactions the macromolecule sample concentration should be at least 5 times the Kd value;
- The buffers used should have low ionization enthalpies (e.g. phosphate, citrate, acetate);
- If the presence of reducing agent is required for a protein stability, then ß-mercaptoethanol (at a concentration lower than 5 mM) or TCEP (lower than 2 mM) should be used rather than DTT.

Differential Scanning Calorimetry (DSC)

The MicroCal VP-DSC instrument measures the temperature of thermally induced structural transitions of molecules in solution. A complete thermodynamic profile is generated to understand the factors that affect conformation and stability of proteins, nucleic acids, micellar complexes and other macromolecular systems.

- The operating temperature range is of -10°C to 130°C;
- Maximum scan rates are 90°C/hr in the upscan mode and 60°C/hr in the downscan mode.

DSC can be used for:

- The determination of transition midpoint, enthalpy (ΔH) of unfolding due to heat denaturation and change in heat capacity (ΔCp);
- The study of factors that contribute to the folding and stability of native biomolecules, including hydrophobic interactions, hydrogen bonding, conformational entropy, and physical environment.

Sample requirements:

- Sample buffer and buffer in the reference cell should be exactly the same;
- The sample solutions should be dialysed against the buffer solution used for the DSC measurements.
- Sample and reference cell volumes are 200 µl;
- Typical sample concentration: 0.2 - 2.0 mg/ml;
- If the presence of reducing agent is required for the sample, the use of up to 5 mM ß-mercaptoethanol or TCEP instead of DTT is recommended;

- Since fluoride-containing samples cause irreparable damage to the VP-DSC cell, their use is prohibited.

Microscale Thermophoresis (MST)

The Monolith NT.115 MST device allows to detect changes in hydration shell, charge or size of molecules and thus to detect biomolecular interactions.

MST can be used for:

- Determination using a fluorescent dye or fluorescent protein of the affinity of interaction from 1nM to mM.

Sample requirements:

- Concentration of fluorescent labeled molecule: 10 nM - 10 mM;
- Final concentration of unlabeled molecule should be at least two orders of magnitude above the expected Kd value. To perform simulations of binding events and to help choose the appropriate concentration, the “Concentration Finder” software is available on the device control panel;
- At least 20 µl samples per capillary is needed.

Label-free MST

The NT.LabelFree MST instrument uses intrinsic tryptophan fluorescence for microscale thermophoresis detection, thereby allowing label-free and immobilization-free experiments.

MST can be used for:

- The label and immobilization free determination of protein binding to Ions, nucleic acids, small molecules and sugars (with an affinity of interaction in the range of 10 nM to mM).

Sample requirements:

- Concentration range of tryptophan-containing protein: 100 nM-10 µM;
- Final concentration of unlabeled molecule should be at least an order of magnitude or more above the expected Kd value;
- Molecular weight range: 10-107 Da;
- Minimum sample volume used: 10 µl per sample.

Differential scanning fluorimetry (DSF)

The Prometheus NT.48 instrument measure native DFS to determine protein thermal transition temperatures and stability of 48 up to samples at a time.

- No dye is required, tryptophan fluorescence at 330 nm and 350 nm is detected;
- Temperature range: from 15 °C to 95 °C.

DFS can be used for:

- Determination of thermal transition temperatures and stability of proteins.

Sample requirements:

- Protein must contain tryptophans in order to detect protein unfolding;
- Sample concentration range: from 5 µg/ml to 250 mg/ml;
- Prepare at least 20 µl of your samples;
- For thermal unfolding experiments no assay development or special sample preparation is needed.

Dynamic/static light scattering

The Zetasizer Nano ZS90 instrument is used for the measurement of particle and molecular size using Dynamic Light Scattering, with the option of measuring zeta potential and electrophoretic mobility, and molecular weight using Static Light Scattering.

- Size (diameter): from 0.3 nm to 5 microns;
- Molecular weight measurement down to 10 kDa;
- Temperature range 0-90°C.

Established methodologies and provided services:

- Particle size analysis (hydrodynamic radius);
- Temperature range 0-90°C.

Sample requirements:

- 25 µl of sample and the same volume of “empty“ buffer;
- For protein solutions, concentrations of at least 0.2 mg/ml;
- For the measurements of zeta potential in folded capillary cells, 0.75 ml of sample is required.

The Zetasizer Ultra is an instrument for the fast and accurate particle and molecule sizing, particle charge (zeta potential) and particle concentration measurements and represents the most intelligent and flexible instrument in the Zetasizer range.

- Dynamic light scattering (DLS) with Non-Invasive-Back-Scatter (NIBS) - particle size;
- Multi-Angle Dynamic Light Scattering (MADLS) - particle size and concentration;
- Electrophoretic light scattering (ELS) - zeta potential, electrophoretic mobility;
- Static light scattering (SLS) - particle weight.

Established methodologies and provided services:

- Particle size analysis (hydrodynamic radius);
- Temperature range 0-90°C.

Sample requirements:

- Non-Invasive Back-Scatter (NIBS) (173°) - 0.1 mg/ml;
- Forward angle (13°) - 5 mg/ml;
- Side angle (90°) and Multi-Angle Dynamic Light Scattering (MADLS) - 1 mg/ml.

Photoluminescence Spectrometry

The FLS1000 Photoluminescence system is a modular fluorescence spectrometer for measuring spectra from the ultraviolet to the mid-infrared spectral range (up to 5,500 nm), and lifetimes spanning from picoseconds to seconds.

Steady State Fluorescence can be used to determine:

- Excitation and Emission Scans
- Steady State and time-resolved Fluorescence Anisotropy
- Kinetic Measurements
- Excitation-Emission and Temperature Maps
- Absolute Quantum Yield Measurements
- Steady State Singlet Oxygen Emission

Time-Resolved Fluorescence can be used to determine:

- Single and Multiple Exponential Decays
- Time-Resolved Emission Spectroscopy (TRES)
- Monomer-Excimer Kinetics
- Time-Resolved Fluorescence Anisotropy
- Solvent Relaxation Dynamics

Data collection:

- Steady-state fluorescence spectroscopy investigates the long-term average fluorescence of a sample when irradiated with UV, Visible or near-IR Light.
- Time-resolved fluorescence (or fluorescence lifetime) spectroscopy is an extension of steady-state fluorescence. Fluorescence lifetimes, occurring as emissive decays from the singlet-state, can also be approximated as those decays occurring in the time region from picoseconds to nanoseconds.

Fourier-transform Infrared (FTIR) Spectrometry

The Vertex 70v spectrometer offer unmatched performance and versatility for demanding analytical and research applications in FTIR. The data acquisition is based on two-channel delta sigma analog-to-digital converter with 24-bit dynamic range, which are running in parallel and integrated into the detector pre-amplifier electronics. This advanced DigiTect technology prevents external signal disturbance and guarantees the highest signal-to-noise ratio. Device provides the unique possibility to acquire a complete far and mid IR spectrum from 6000 cm-1 to 50 cm-1 in a single step measurement.

FTIR System with:
Flow through transmission cell AquaSpec can be used for:

- Protein stability measurement
- Quantification structural changes / denaturation
- Secondary structure determination
- Concentration determination
- Protein-protein and protein-ligand interaction characterization

Bio ATR II cell can be used for:

- Temperature ramps
- Aggregation processes
- Membrane proteins study

Mass photometry

The Two MP instrument is used for the determination of the molecular mass and the oligomerisation of a sample in their native state. Technology can detect and characterise proteins, nucleic acids, lipids and sugars. It provides information on structure, homogeneity, and function (quantifying interactions) – all in a matter of minutes and using tiny amounts of sample.

- Measurement of molecular mass across a wide mass range, from 30 kDa up to 5 MDa.

Sample requirements:

- Sample concentration: nM range
- Quantity: 20 µl

Microplate reader

The SPARK microplate multimode reader offers absorbance (from 200 to 1 000 nm), fluorescence, including FRET and fluorescence polarization, and luminescence (ex 230 – 900 nm, em 280 – 900 nm) measurements. Technique provides a solution for ELISAs, low volume DNA/protein quantification and fast spectral scanning. Plate reader has a higher performance, sensitivity and flexibility.

Sample requirements:

- Cuvettes and microplate formats up to 1 536 wells.

Bio-layer Interferometry

Octet R8 instrument enables label-free analysis for the determination of kinetics and affinity of biomolecular binding using bio-layer interferometry. System is a fluidics-free and low maintenance detection. Eight parallel, independent channels provide maximum speed, sensitivity and flexibility.

- The Octet R8 system monitors binding events in real time to calculate on rates (ka), off rates (kd), and affinity constants (KD).
- The sample plate temperature can be controlled from 15–40°C.
- Different sensor types, including streptavidin, anti-His, Ni-NTA, anti-human IgG biosensors are available.
- Analysis can be done using a single channel or up to eight channels

Sample requirements:

- Analysis of up to 96 samples in microplate wells.
- Load Sample (immobilized) 10-50 µg/ml (~µM range)
- Analyte 0.01 – 10xKD

HeliX switchSENSE dynamic biosensors technology

is based on customizable DNA nanolevers on a chip surface. Different measurement modes allow a multiparameter characterization of biomolecules. All measurement modes are based on changes in the fluorescent intensity of a dye attached to the nanolever. The fluorescence depends on the local environment of the dye as well as the distance of the dye from the quenching gold electrode surface of the heliX chip.

In static mode, the nanolever itself is not moving. The static mode detects the binding of molecules in real-time through changes in the dye's local environment (*fluorescence proximity sensing*). The static measurement mode is also applied when assessing the enzymatic activity of nucleic acid modifying enzymes. These assays exploit the quenching effect of the gold surface.

In the dynamic measurement mode, the DNA nanolevers are electrically actuated to oscillate at high frequencies. The measured instantaneous velocity of the nanolever depends on the hydrodynamic friction of the attached molecules. Thus, changes in the upward motion of the nanolever can be used to determine ligand size and shape. Larger molecules or conformational changes that cause an expansion of the molecule will slow down the movement, whereas smaller molecules or analyte-induced compaction of the molecule will increase the velocity of the motion.

Sample requirements:

The heliX⁺ biosensor is equipped with 4 single-photon counters for highest fluorescence sensitivity as well as for the detection of two different fluorophores. Therefore, two independent signals from two interactions can be monitored at the same time and on the same sensor spot. This allows for many different assay possibilities like multiplexing, bispecific analytes and energy transfer assays (e.g. FRET).

The heliX chips have two sensor spots. By using the possibility of measuring two independent interactions simultaneously on the same sensor spot, four signals can be monitored in parallel, allowing for real-time referencing of two interactions at the same time or multiplexing of up to four interactions per run.

proFIRE is a unique system for protein-DNA conjugate preparation.

DNA length 10 – 150 nt
Protein molecular weight 5 – 500 kDa
Injection volume 50 – 150 µL
Number of fractions 12
Fraction volume 200 – 800 µL
Temperature range 10 – 30 °C

7. Crystallization and Diffraction

Core Facility contacts: **Jiří Pavlíček**, jiri.pavlicek@ibt.cas.cz
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Instruments available:

- Gryphon dropsetter (ArtRobins) – multi-channel (96-channel) pipetting robot for easy setup of nanodrop crystallization plates (20 °C)
- Dropsetters NT8 (Formulatrix) – automated crystallization robots with controlled humidity, LCP capability, and robotic seeding (10 °C and 20 °C)
- Crystallization hotel RI1000 (Formulatrix) with SONICC detection – automated storage (20 °C) and monitoring of crystallization plates with remote image access
- Crystallization hotel RI182 (Formulatrix) – automated storage (10 °C) and monitoring of crystallization plates with remote image access
- Glovebox with stereomicroscope (GS) – crystallization and crystal manipulation under controlled (oxygen-free) atmosphere
- NanoDLS SpectroLight 600 (Xtal Concepts) – in-drop dynamic light scattering (DLS) measurements
- Vitrobot (Thermo Scientific) – vitrification of samples for cryo-EM and electron diffraction
- D8 Venture diffractometer (Bruker):
  - Single-crystal diffractometer with high-flux liquid gallium MetalJet D2+ X-ray source, Photon III detector, and kappa goniometer
  - High-resolution measurements under cryogenic conditions
  - ISX stage – in situ diffraction screening directly in crystallization plates using a motorized stage
  - HC-Lab (Arinax) – controlled humidity environment for crystal dehydration and room-temperature experiments
  - XRF – elemental analysis using X-ray fluorescence
- SAXSpoint 2.0 (Anton Paar):
  - SAXS/WAXS measurements
  - Equipped with MetalJet D2+ X-ray source and Eiger 1M detector
  - Automated sample delivery
  - In-situ UV-Vis spectrometer (Agilent)
  - Liquid chromatography system (ÄKTA Go, GE Healthcare) for SEC-SAXS

Services provided:

All services are available to both inexperienced and advanced users. Consultation with facility staff is recommended prior to starting experiments.

Crystallization

- Robotic setup of 96-well crystallization plates for screening and routine crystal production (proteins, nucleic acids, and complexes)
- Manual setup of crystallization experiments for screening and optimization using various plate formats
- Crystallization at controlled temperatures: 10 °C and 20 °C
- Crystallization under inert atmosphere using a glovebox
- Automated monitoring of crystallization in Formulatrix hotels (10 °C and 20 °C), including visible, polarized, UV, and SONICC (SHG and UV-TPEF) detection
- Remote access to crystallization images with automated preliminary evaluation

Sample Preparation

- Crystal harvesting, cryo-protection, and cryo-cooling (liquid nitrogen vitrification)
- Long-term storage of crystals in liquid nitrogen
- Vitrification of samples for cryo-EM and electron diffraction
- Sample preparation under anaerobic conditions
- Supply of cryo-loops and EM grids

X-ray Diffraction

- Diffraction screening of macromolecular crystals
- In-house data collection and processing
- In situ diffraction evaluation of sensitive samples
- Experimental phasing (MIR, SAD, MAD)
- Elemental analysis (XRF)

SAXS

- Small-angle X-ray scattering (SAXS/WAXS) measurements
- Structure analysis and sample quality assessment
- SEC-SAXS experiments (online or offline chromatography)

8. Structural mass spectrometry

Core Facility contact: **Petr Pompach**, petr.pompach@ibt.cas.cz
Jan Rasl, jan.rasl@ibt.cas.cz
Geovanna de Oliveira Costa, geovanna.deOliveiraCosta@ibt.cas.cz

Instruments available:

- 15T-Solarix XR FT-ICR mass spectrometer with electrospray and MALDI ion source (Bruker Daltonics);
- Autoflex Speed MALDI-TOF mass spectrometer (Bruker Daltonics)
- timsTOF Pro mass spectrometer (Bruker Daltonics)
- timsTOF SCP mass spectrometer (Bruker Daltonics)
- 1290 UPLC system (Agilent Technologies)
- 1290 bioinert UPLC system (Agilent Technologies)
- Evosep One HPLC system (Evosep)
- Excimer laser (Coherent)
- UV ExciStar laser (Coherent)

Services provided:

All services at CMS (Centre of Molecular Structure of Biocev) are available both to unexperienced users and to experienced users (that do not require the assistance of the scientist in charge)

- Protein molecular weight determination by ultra-high resolution 15T FT-ICR mass spectrometer with sequence confirmation by Top-down approach using different fragmentation techniques (collision induced dissociation, electron transfer/capture dissociation, infrared multiphoton dissociation);
- Shotgun proteomics – identification and quantification of proteins/peptides.
- Characterization of posttranslational modification such as phosphorylation, glycosylation or disulphide bonds;
- Structural mass spectrometry: limited proteolysis, hydrogen/deuterium exchange, chemical cross-linking, covalent labelling and native mass spectrometry with ion mobility separation (timsTOF Pro and timsTOF SCP);
- Fast Photochemical Oxidation of Proteins (FPOP) - a type of hydroxyl-radical-based protein footprinting;
- Metabolomics – identification and quantification of metabolites;
- Processing and interpretation of mass spectrometric data;

9. Protein Production

Core Facility contact: **Miroslava Alblova**, miroslava.alblova@ibt.cas.cz
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Instruments available:

- Biometra TAdvanced Twin PCR Thermal Cycler (Analytik Jena)
- Azure 300 Imaging System (Azure Biosystems)
- Innova® 44R Incubator Shaker (New Brunswick)
- Ecotron Incubator Shaker (INFORS HT)
- Biohazard box SafeFAST Classic S/D (class II, FASTER)
- Innova® 40/40R Benchtop Orbital Shaker (New Brunswick)
- S41i - CO_2 Incubator Shaker (New Brunswick)
- IncuSafe CO_2 static incubator MCO-170AIC (PHCbi)
- Celltron shaker for CO_2 incubators (INFORS HT)
- CellDrop FL Unlimited cell counter (DeNovix)
- ECLIPSE Ts2 FL inverted microscope (Nikon)
- Avanti J-26S XP High-Speed centrifuge (Beckman Coulter)
- Q700 Sonicator (QSONICA)
- ÄKTA start protein purification system (Cytiva)
- NGC Chromatography System (Bio-Rad)
- Superdex 75 or 200 columns (10/300 increase or HiLoad 16/600, Cytiva)
- Trans-Blot® Turbo™ Transfer System (Bio-Rad)
- DS-11+ Spectrophotometer (DeNovix)
- Lyophilizer L10-55 PRO (GREGOR Instruments)

Services provided:

The Protein Production core facility of CMS (Centre of Molecular Structure of the Institute of Biotechnology, Biocev) provides preparation of expression vectors and all steps of recombinant protein production in *E. coli* or in eukaryotic expression systems, as well as the subsequent purification of the corresponding proteins.

Cloning services

- Checking or preparation of the DNA templates – design of template and its synthesis in form of a DNA string by a specialized company (if the DNA template is not available)
- Plasmid DNA construct preparation – PCR of target gene, preparation of plasmid, ligation, transformation and sequencing
- Cloning using traditional restriction enzymes or restriction-free methodology
- Mutagenesis – site-directed mutagenesis of target genes or existing construct and sequencing

Recombinant protein production in *E. coli*

- Small scale expression tests – transformation of DNA constructs into *E. coli* cells, protein expression cultivation in LB medium, cell harvesting and SDS-PAGE analysis (sodium dodecyl sulphate – polyacrylamide gel electrophoresis)
- Small scale solubility tests – solubilization of samples using sonication, separation of soluble fractions and SDS-PAGE analysis
- Large scale protein expression in *E. coli* expression systems – overnight or day culture (max. 4 hours after induction) in LB medium (Luria-Bertani) or in other medium (for example production of labelled proteins in minimal media)
- Different bacterial strains are available: BL21 (DE3), SHuffle T7, Arctic Express (DE3), Rosetta, DH5 alpha, Lemo21(DE3), etc.

Recombinant protein production in insect cells

- BacPAK™ (TaKaRa) baculovirus systems
- Small-scale cell transfection and expression verification by Western blot
- Baculovirus amplification
- Large-scale production in Sf9 or High Five™ cells

Recombinant protein production in mammalian expression systems

- Expression in HEK293T/17SF, Expi293™, Expi293F™ GnTI- cells
- Small-scale cell transfection and expression verification by Western blot
- Large-scale cell transfection and protein production

Recombinant protein purification

- Cell lysis by sonication and separation of soluble fraction
- Affinity chromatography (HiTrap HP, Ni-INDIGO, Strep-Tactin®XT, GST, cobalt) on FPLC or by gravity flow
- Protein concentration and dialysis
- Tag cleavage (TEV, SUMO, 3C protease)
- Ion-exchange chromatography – HiTrap Q HP anion exchange chromatography column or HiTrap SP HP cation exchange chromatography column (1 or 5 ml)
- Size exclusion chromatography (SEC) – Superdex 75 or 200 (10/300 increase or HiLoad 16/600) columns
- Protein purity, concentration and identity testing – SDS-PAGE (homemade or 8 – 16 % gradient gels from Bio-Rad), testing by Bradford Dye, determination of protein concentration

using DeNovix spectrophotometer, native PAGE (polyacrylamide gel electrophoresis), identification of purified proteins by mass-spectrometry (MS)

All DNA constructs are provided with the final protocol and sequencing results. Proteins samples can be verified using MS in another CMS core facility. Customizations and optimizations of our standardized protocols are possible and preparations according to customers established protocols.