CIISB

Czech Infrastructure for Integrative Structural Biology

Equipment and services of CIISB research infrastructure available for **2016 call** for proposals

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MINISTRY OF EDUCATION, YOUTH AND SPORTS

All Core Facilities accessible through CIISB research infrastructure are part of Czech National Affiliated Centre of Instruct.

Content

1. Biomolecular Interactions and Crystallization

Core Facility contact: **Michaela Wimmerová**, bic@ceitec.cz

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

- General information, methodology selection, details: michaela.wimmerova@ceitec.muni.cz
- Calorimetry: jitka.zdanska@ceitec.muni.cz
- SPR: lenka.malinovska@ceitec.muni.cz
- AUC: jan.komarek@ceitec.muni.cz
- MST/DSF: jitka.zdanska@ceitec.muni.cz, josef.houser@ceitec.muni.cz
- Crystallization and structural studies: josef.houser@ceitec.muni.cz

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

Instruments available:

Isothermal Titration Calorimetry (ITC)

- ITC method is used for characterization of biomolecular interactions of small molecules, proteins, antibodies, nucleic acids, lipids and others.
- Enzyme kinetics, biological activity or the effect of molecular structure changes on binding mechanism can be also assessed.
- Complete thermodynamic profile of the molecular interaction in a single experiment (stoichiometry, K_a, ΔH and ΔS values) or kinetics parameters K_m and k_{cat} can be determined.
- **Auto-iTC200** and **VP-ITC** are designed to measure the heat of binding. In a typical arrangement, the titrant, also referred as the ligand, is injected into the sample cell containing the macromolecule sample material.
- The calorimetric measurement can be done over a range of biologically relevant conditions (temperature, salt, pH, etc.).
- ITC system directly measures submilimolar to nanomolar binding constants (10^3 - 10^9 M^{-1}). Interactions with nanomolar to picomolar binding constants (10^9-10^{12} M^{-1}) can be measured

using the competitive binding technique, the same principle can be used for low affinity interactions (10^3-10^2 M^{-1}). The operating temperature range is of 2°C to 80°C.

- Two operation modes are available: user self-operating measurement (VP-ITC) or performing the measurement by core facility staff (Auto-iTC200).

Established methodologies and provided services:

- Calorimetric measurement of protein-ligand interaction (Standard titration method, Single injection method)
- Competitive-based measurement - low or high affinity interactions
- Data evaluation - thermodynamic parameters determination using curve fitting models: One set of binding site, Two sets of binding sites
- Eventuality of manual data evaluation using fitting models: Sequential binding sites, Competitive binding, Dissociation

Data processing:

- Using the Origin software and NITPIC (possibility to train people in data processing)

Sample requirements - importance of sample preparation

- Proper sample preparation is crucial for the successful ITC measurement. The buffer solution, in which the macromolecule and the ligand are dissolved, should be exactly the same. Concentration of samples must be determined precisely.
- The macromolecule sample (the sample placed in the cell) must have a volume **at least 450.0 µl for Auto-iTC200** and **1800.0 µl for VP-ITC calorimeter**. Preferably, the macromolecule solutions should be dialysed against the buffer solution used for the ITC measurement. A lyophilized macromolecule sample devoid of salts or additives may be dissolved directly into the buffer, the pH should be checked before the measurement.
- The ligand solution (the sample placed in the injection syringe) must have a volume at least **150.0 µl for Auto-iTC200** and **500.0 µl for VP-ITC calorimeter**. Generally a concentration of ligand should be 10 times higher than the concentration of macromolecule.
- High affinity interactions can be studied at low concentrations. In this case the minimum concentration of macromolecule sample which causes measurable heat is 10 µM. For low affinity interactions the macromolecule sample concentration should be 5 times of K_d or higher, but higher concentration may be limited by availability or solubility of samples.
- Calculating the cell sample concentration - **$M = c / (n \times K_a)$**
- c-value … (should lie between 10-500); n … binding stoichiometry;
- M … molar concentration of the cell sample; K_a …association constant; K_d … dissociation constant
- At least 10 ml of the used buffer must be sent for each measurement (for Auto-iTC200).
- If possible, choose a buffer with low ionization heat in order to minimize artificial heats of buffer ionization (e.g. phosphate buffer works well).

- If the presence of reducing agent is required for a protein stability, then ß-mercaptoethanol (less than 5 mM) or TCEP (less than 2 mM) should be used rather tan DTT.

Circular Dichroism (CD) spectroscopy

- **CD spectropolarimeter Jasco J-815** - This instrument can measure: circular dichroism, fluorescence, total fluorescence, linear dichroism, magnetic dichroism, optical rotation dispersion, and stopped flow circular dichroism, fluorescence and absorbance. Circular dichroism and fluorescence data can be acquired simultaneously. Wavelength range 185 – 900 nm. All the measurements on JASCO-J815 are done in 1 – 10 mm cells according to buffer composition and protein concentration.
- Accessories:
  - Peltier temperature control
  - Monochromator for fluorescence
  - Bio-Logic: SFM-20; two channel stopped-flow setup

Circular dichroism can be used:

- for the determination of protein folding
- to characterize protein`s secondary structure
- to detect the changes in structure upon mutagenesis
- to study conformational stability of proteins (pH stability, denaturant stability, temperature, buffers addition of stabilizers) or
- to detect the changes in the conformation of a protein upon protein-protein interaction

Data collection:

- Wavelength scanning:
  - Continuous scan: running average method offering high speed measurements
  - Step Scan: discrete wavelengths and response time to optimize signals
  - Auto-scan: based on step scan but offering a range of response times to speed data acquisition
- Time scan
  - Fixed wavelength time scan for chemical denaturation and stopped-flow experiments
- Temperature scan
  - Fixed wavelength for CD vs. Temperature thermal ramping
  - Pre-set temperatures with equilibration times for spectral scanning
  - 3 Dimensional display of CD vs. Wavelength vs. Temperature or Time

Data processing (possibility to train the people how to analyse the data):

- JASCO's new - Spectra Manager system
- DICHROweb
- K2D3

CD Spectroscopy requirements:

Far-UV CD spectra (secondary structure measurement) require between 300 µl - 700 µl of ~ 0.1 – 0.5 mg/ml protein solution, in any buffer, which does not show a high absorbance in this region of the spectrum. Substances not optimal for CD: DTT or stabilizing salts (high concentrations only), imidazole, Triton X-100.

Analytical ultracentrifugation (AUC)

- **Beckman Coulter ProteomeLab XL-I** - The instrument is equipped with absorbance optics (wavelength range 190 – 800 nm) and interference optics and can be used for both sedimentation velocity and sedimentation equilibrium experiments. Analytical ultracentrifugation has a broad applicability in science including determination of sample homogeneity, oligomeric state of proteins (or molecular weight, respectively) and can be used to assess aggregation and to study biomolecular interactions of self- and hetero-association systems (determination of stoichiometry, determination of affinity in the range of 10^4-10^8 M^{-1})
- Accessories:
  - four hole An-60 Ti rotor
  - quartz and sapphire windows
  - flow-through double sector centerpiece cells for sedimentation velocity experiments
  - six-channel cells for sedimentation equilibrium experiment
  - additional cells for special purposes available

Established methodologies and provided services:

- Sedimentation velocity - determination of sedimentation coefficient, assessing sample heterogeneity, determination of oligomeric state of proteins, detection of aggregation in the sample, study of biomolecular interactions
- Sedimentation equilibrium - determination of molecular weight, study of biomolecular interactions
- Data analysis

Sample requirements:

- both a sample and a reference buffer are required – samples should be equilibrated into the experimental buffer by dialysis or size-exclusion/desalting chromatography (crucial especially for the use of interference optical system)
- buffer (usually 10-20 mM): buffers should not absorb at a wavelength where the sample is measured (e.g. phosphate buffers work well, TRIS and HEPES are tolerable at low concentrations for 280 nm)
- ionic strength (at least 100-200 mM NaCl, or even higher for highly charged proteins): sufficient ionic strength is needed to prevent electrostatic interactions that would affect sedimentation process

- if possible substances generating density gradients (glycerol, sucrose, cesium chloride) should be avoided
- if the use of reductants (DTT, β-mercaptoethanol) is necessary, they should be used at low concentrations
- concentrations: dependent on absorbtivity, but usually not higher than 1 mg/ml (for proteins)
- volumes:
  - for SV experiment usually 450 µl of both sample (optimal loading absorbance 0.5-1.0 OD for absorbance optics, optimal loading concentration >0.1 mg/ml for interference optics) and reference is required
  - for SE experiment: at least 95% purity of a sample, usually 150 µl of both the sample (optimal loading absorbance 0.2-0.5 OD) and the reference

These requirements depend on the nature of experiments and a particular protein of interest. If the proposal is accepted, they will be further discussed.

Differential Scanning Calorimetry (DSC)

- **VP-DSC** – differential scanning microcalorimeter – measures heat changes that occur in the sample (biomolecule solution) during a controlled increase or decrease in temperature, on the basis of a temperature difference between the sample and the reference material.
- It is a valuable technique for the study of samples in solution providing fast and accurate determination of the transition midpoint T_m – when 50% of the biomolecule are unfolded.
- In addition, a complete thermodynamic profile is generated to understand the factors that affect conformation and stability - enthalpy (ΔH) of unfolding due to heat denaturation, also the change in heat capacity (ΔC_p) of denaturation can be determined.
- DSC is a sensitive, easy-to-use technique that requires no assay development, labelling or immobilization. Filling of the cell is crucial for the accuracy.
- The operating temperature range is of -10°C to 130°C.
- Scanrates fall in the range of 0°C/hr to 90°C/hr in the upscan mode and 0°C/hr to -60°C/hr in the downscan mode. Experiment at constant temperature (Isoscan) for shelf life studies or evaluating of the compound stability is also possible.

DSC can be used:

- for characterization of the stability of proteins or other biomolecules, for elucidation the factors that contribute to the folding and stability of native biomolecules, including hydrophobic interactions, hydrogen bonding, conformational entropy, and the physical environment.
- for characterization of membranes, lipids, nucleic acids and micellar systems. Assessment of the effects of structural change on a molecule's stability - protein engineering or antibody domain studies.

Data collection:

- Conventional DSC - mode uses a linearly increasing or decreasing temperature ramp function, while measuring the differential.
- Isothermal Scan Mode – a constant temperature is maintained for a relatively long period of time while measuring the differential power between the reference cell and sample cell.
- Proper sample preparation is crucial for the successful DSC measurement. Sample buffer and buffer for filling the reference cell should be EXACTLY the same.
- The sample solutions should be dialysed against the buffer solution used for DSC measurement, if it is possible. The pH should be checked before the measurement.
- Volume of sample for filling the sample cell and buffer for filling the reference cell must have at least **800.0 µl**, typically 1.0 ml is recommended.
- If the reducing agent is needed in the sample, usage of up to 5 mM β-mercaptoethanol (or TCEP) instead of DTT is recommended.
- Fluoride compounds can cause irreparable damage of the VP-DSC cell, therefore it is not possible to measure samples containing fluorides.
- Precipitation and aggregation can cause a rapid downward shift or an increase in baseline noise after the system unfolds. Minimizing precipitation is necessary for accurate result.

Microscale Thermophoresis (MST)

- Microscale thermophoresis is based on physical principle of measuring changes of the mobility of molecules in microscopic temperature gradients. Movement of molecules along the temperature gradient is called thermophoresis. This method allows to detect changes in hydration shell, charge or size of molecules and thus detect biomolecular interaction.

MST can be used for:

- to determine the affinity of interaction from 1nM to mM

Sample requirements - importance of sample preparation

- Concentration of fluorescent labeled molecule: 10 nM - 10 mM
- Final concentration of unlabeled molecule should be at least an order of magnitude or more above expected dissociation constant (K_D). For simulation of binding event and for choosing the right concentration there is “Concentration Finder” software available instrument’s control unit.
- **Prepare at least 20 µl of your samples**. Evaporation or sticking of the sample to the microtube’s walls may occur.
- **Spin your samples** – both labeled and unlabeled molecules for 5 min with 13 000 rpm

Differential scanning fluorimetry (DSF)

- The Prometheus uses advanced UV-LEDs for excitation of fluorophores. Tryptophan fluorescence is collected by proprietary UV-detection unit with the ability of rapid and sensitive acquisition of tryptophan fluorescence. The Prometheus detects changes in fluorescence of the amino acid fluorophores (for example tryptophan) over wide range of temperatures. DSF is used to determine **thermal unfolding transition temperatures** and **denaturation midpoints**.
- The instrument can also induce **thermal** or **chemical unfolding of proteins**. For thermal unfolding experiments no assay development or special sample preparation is needed. In case of chemical experiments it is necessary to prepare series of samples with different concentrations of denaturant mixed with your protein and then incubated for equilibration.

DSF can be used:

- to determine thermal unfolding transition temperatures
- to determine denaturation midpoints
- to induce thermal or chemical unfolding of proteins

Sample requirements - importance of sample preparation

- protein must contain **at least one tryptophan** (or other fluorescent residue) in order to detect protein unfolding
- capillary are filled automatically after dipping in to the sample by capillary forces
- **Prepare at least 20 µl of your samples**. Evaporation or sticking of the sample to the microtube's walls may occur

Surface plasmon resonance (SPR)

- **Biacore T200, SPR Imaging multichannel system** exploit the phenomenon of surface plasmon resonance to monitor the interaction between molecules in a real time. One of the interactants is immobilized on the sensor chip surface, while the other is passed over that surface in solution.
- Applications of SPR include biotherapeutic and drug discovery research, as well as protein activity and stability analysis in biopharmaceutical production. SPR is suitable also for characterization of membranes, lipids, nucleic acids and micellar systems.
- **Biacore 3000** enables measurement on up to four channels in one run. Various types of sensor chips are available - gold layer, hydrophobic layer, NTA for metalo-affinity interaction or carboxymethylated for covalent immobilization of biomolecules.
- **SPR Imaging multichannel system** enables to immobilize 5 different binding partners on the sensor chip surface at the same time. 5 different ligands can be passed over these 5 different immobilized binding partners.

SPR can be used for:

- Test of protein activity
- Specificity - searching for binding partners, characterization of inhibitors affinity, test for cross-reactivity**,** eventually directly to test expression of a given protein in cell line cultures
- Affinity (kinetics) – kinetic and equilibrium parameters of an interaction, the rates of complex formation (k_a), dissociation (k_d), and equilibrium association/dissociation constants can be determined.
- Concentration determination - concentration is determined by monitoring the interaction of a molecule with a prepared sensor surface in the presence of a target molecule in solution (solution inhibition) or excess analyte (surface competition).
- Multiple interaction during complex formation - complex formation can be monitored as each component is incorporated into a multimolecular complex.

Data collection:

- Direct binding assay - measure the amount of analyte bound directly to the detecting molecule after sample injection
- Binding rate measurement - monitoring of complex formation continuously as a function of time.
- Indirect or competition (inhibition) assays - known amount of detecting molecule is mixed with sample, and the amount of free detecting molecule remaining in the mixture is measured.

Sample requirements - importance of sample preparation

- Sample should be filtrated through 0,2 µm filter as well as a running buffer.
- Sample environments that differ greatly from the running buffer will give rise to a bulk refractive index (RI) effect that is commonly present during an injection. Bulk refractive index effects do not affect the binding but could hide the interaction. The recommendation is that the samples should be diluted in a running buffer to minimize bulk effects or preferably to use the sample buffer as a running buffer if possible. On-line reference subtraction helps to minimize the effects of bulk.
- 50 µl sample at least is needed for one measurement (depends on method set-up).
- Most of the buffer compound is possible to use, 70% of ethanol and higher conc. is not allowed.
- Immobilization of one interacting partner is essential. Choose wisely the sensor chip that will be used for immobilization of your sample. If you are in doubt, ask for expert consulting on site to minimize the risk of chip degradation.

Dynamic/static light scattering

- **DelsaMax Core** Dynamic/static light scattering (DLS/SLS) - is used for the analysis of protein solutions, aggregates, promiscuous inhibitors, buffers, nanoparticles, polymers or other products in solution.

- DLS can measure the polydispersity of your sample and hydrodynamic radii (size) of the particles. This is particularly beneficial in sample characterization prior to crystallization or other experiments.
- Broad spectrum of particle sizes can be analysed (hydrodynamic radius range of 0.2 to 2,500 nm)
- In static light scattering (SLS) the average molecular weight of a particle in solution can be obtained (range of 300-10^6 Da, concentration-dependent)

Established methodologies and provided services:

- Sample polydispersity analysis
- Particle size analysis (hydrodynamic radius)
- Molecular weight determination
- Data analysis
- Training in data analysis

Sample requirements:

- **20 µl** of each sample required, the same volume of buffer without studied particle is recommended
- For protein solutions, **0.1 mg/ml** concentration and higher is recommended.
- Method accessible range is 0.4 to 5,000 nm in hydrodynamic radius, up to 10 MDa in molecular weight (concentration dependent)
- Standard laboratory plastic-compatible buffers are suitable
- For particle size determination high monodispersity (homogeneity) of the sample is strongly recommended.

Protein Crystallography:

- Automatic liquid handling systems - Offer an advanced, proven and reliable liquid handling system for different scales and throughputs. The instruments can automate a diverse range of applications including primary and secondary screening for protein crystallization.
- Crystallization robots for setting-up of screening and optimization plates – **Mosquito, Dragonfly** - Usage of nanoliter volumes of protein sample results in cost savings and allowing more extensive screening. Mosquito automates protein crystallization techniques; sitting drop, microbatch as well as seeding or additive screening plate preparation and is also capable of working with liquid cubic phase (LCP). The accuracy and repeatability means that the drops are placed centrally in the sub-wells of sitting-drop 96-well plates every time. Dragonfly screen optimiser enables complex assay gradients or optimisation screens to be rapidly and accurately prepared in 96-well gradient plates. Once the initial crystal ‘hits’ are identified, dragonfly optimizes the set of conditions to grow better diffracting crystals.
- Automated Minstrel HT UV Crystal Imaging System - The Minstrel HT UV is an ultraviolet and visible crystal imaging and protein crystal monitoring system. It automatically images

crystallization experiments and links images with crystallization conditions. Its UV technology can find crystals in complex drops and easily distinguish protein crystals from non-protein crystals (such as salt).

- Accessories:
  - 96-well UVP screening plates (possibility of 3 sitting drops) and 24-well optimization plates (sitting drop or hanging drop)
  - Commercial screens (over 2000 possible conditions including membrane proteins):
    - Qiagen: AmSO4 Suite, Classics Suite, Classics II Suite, Classics Lite Suite, ComPAS Suite, Cryos, PACT Suite, PEGs Suite, PEGs II Suite, pHClear Suite, pHClear II Suite, Protein Complex
    - Molecular Dimensions: Structure I+II Screen, MemStart & MemSys, Morpheus
  - Automated Minstrel HT UV for inspection of screening plates
  - Leica microscope with polarizing filter
  - Temperature optimizer for crystallization TG40 for 5 different temperatures
  - Possibility to set-up screening and optimization screens at 4 different temperatures (4, 12, 17 and 22 °C), the crystal imaging at 4 and 20°C

Established methodologies and provided services:

- Protein purity analysis by dynamic light scattering
- Thermal shift assay – thermal denaturation assay that measures the thermal stability of a target protein under the certain conditions.
- Set-up of crystallization screens (drop volume 200 nl) using commercial kits (including kits for membrane proteins and protein complexes)
  - Sitting drop crystallization method
  - Crystallization under oil
- Automatic screening of crystals
  - Regular automatic inspection within the period of one month
  - UV imaging to distinguish a protein from a salt
- Storage of the screening plates for extended period of time (6 months) at a constant temperature (4°C, 20°C) with a possibility of a demanded extra inspection
- Setting-up multidimensional gradients for optimisation (from up to 5 different solutions)
- Set-up of optimization screens
  - Hanging drop crystallization method
  - Sitting drop crystallization method
  - Crystallization under oil
- Estimating of the precipitation diagram
- Production of crystals for structural determination
- Improvement quality of crystals for structural determination

Training people in further data processing

- Using popular packages such as MOSFLM and XDS
- Structure determination using MR, MIR, MAD, SAD, techniques and software packages such as CCP4, CNS and SHELX
- Structure refinement and fitting using Refmac and Coot
- Structure visualization and analysis using PyMol and VMD

Protein crystallography requirements:

Between 300 µl - 500 µl of ~ 10 mg/ml protein solution in any buffer (depending on numbers of screening conditions – at least 30 µl per plate). Detailed information about the buffer solution required. For protein crystallography, sample purity is crucial. Therefore a picture of SDS-PAGE gel to check the purity of the sample is required

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2. X-ray Diffraction and Bio-SAXS Core Facility

Core Facility contact: **Jaromír Marek,** jaromir.marek@ceitec.muni.cz

Instruments available:

- Robotized macromolecular diffraction system Rigaku HighFlux HomeLab™ with sample changer ACTOR optimized for work at Cu-K_α wavelength
- Universal, dual wavelength (Mo-K_α and Cu-K_α) diffractometer Rigaku HighFlux HomeLab™
- SAXS camera Rigaku BioSAXS-1000 for small angle X-ray scattering from solutions of biological macromolecules or from nanostructures

Services provided:

- Basic characterization of solution of biological macromolecules by SAXS
- Determination of a low resolution 3-D shape of biological macromolecules by SAXS
- SAXS characterization of non-biological nanostructures
- Test of a diffraction quality of protein crystals, derivatives, cryoprotectants etc prior data collection
- Collection of diffraction data with crystals of biological macromolecules
- Data collection and solving of the crystal structures with non-biological single crystals
- Collection of high angle diffraction data with non-biological single crystals
- Collection of diffraction data with small and/or weekly diffracting non-biological single crystals

3. Nanobiotechnology Core Facility

Core Facility contact: **Petr Skládal**, skladal@chemi.muni.cz

Nanobiotechnology Core Facility provides atomic force microscopy (AFM) services and related experience for imaging and other studies of biological objects, including affinity interactions at the molecular level - force spectroscopy.

Available instruments and techniques:

- scanning probe microscope - Ntegra Vita / Solaris (NTMDT) - exchangeable measuring heads for AFM, STM, SNOM, electrochemical AFM, nanolithography
- atomic force microscope NanoWizzard3 (JPK) mounted on the inverted confocal fluorescence microscope IX81 CLSM FV1200 (Olympus)
- ForceRobot 300 (JPK) - automated force-distance curves for molecular nano-biointeractions
- automated system SolverNEXT (NTMDT) - basic AFM scanning
- fast-scanning AFM for biointeraction studies FastScanBio (Bruker)
- ink-jet based deposition system SciFlex Arrayer S3 (Scienion) - preparation of microarrays

Services provided:

The imaging of biomolecules, cells and other biological structures and objects is realized in aqueous solution or in the dry state. 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. Different scanning tips with appropriate sharpness and cantilevers with a wide range of force constants.

- preparation of samples for AFM – fixation at the support materials
- visualization and studies of samples using atomic force microscopy (AFM) both in dry state and in liquid, imaging using contact mode (static) and dynamic modes as tapping (intermittent contact, amplitude modulation AFM) and non-contact (frequency modulation AFM)
- AFM combined with other techniques: electrochemistry, optical and confocal fluorescence microscopy
- force spectroscopy - automated generation and evaluation of force-distance curves for tips and surfaces modified with complementary biomolecules (e.g. antigen-antibody, biotin-avidin, ligand-receptor)
- nanolithography, nanomechanical manipulations, ink-jet based deposition
- AFM measurements, statistical analysis and data filtration, mechanical, electric and magnetic 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.

4. Josef Dadok National NMR Centre

Core Facility contact: **Radovan Fiala**, radovan.fiala@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 500 MHz – available with room temperature triple-resonance (^{1}H, ^{13}C, ^{15}N) 5 mm probe, 10 mm dual (^{1}H, ^{13}C) probe, nitrogen-cooled multinuclear cryoprobe (Prodigy) and 4.0 mm solid-state dual CP/MAS probe.
- NMR Spectrometer Bruker AVANCE 600 MHz equipped with a quadruple resonance (^{1}H, ^{13}C, ^{15}N, ^{31}P) cryoprobe with -40 to 80^{0}C temperature range.
- NMR Spectrometer Bruker 700 MHz for biomolecular applications, equipped with a triple-resonance (^{1}H, ^{13}C, ^{15}N) cryoprobe optimized for detection of ^{13}C, -40 to 80^{0}C temperature range.
- NMR Spectrometer Bruker 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 (^{1}H, ^{13}C, ^{15}N) probe, 3.2 mm solid-state triple-resonance (^{1}H, ^{13}C, ^{15}N) MAS probe and 4.0 mm solid-state dual CP/MAS probe.
- NMR Spectrometer Bruker 850 MHz equipped with a triple-resonance (^{1}H, ^{13}C, ^{15}N) cryoprobe, 0 to 135^{0}C temperature range.
- NMR Spectrometer Bruker 950 MHz equipped with a triple-resonance (^{1}H, ^{13}C, ^{15}N) cryoprobe, -40 to 80^{0}C temperature range.

Services provided:

Besides providing the access to the spectrometers, the core facility offers consultations on the choice of suitable NMR measurements, support in setup and running the experiments, processing the data and evaluation of the results. The facility can run the latest multidimensional measurements using non-uniform sampling methods and direct carbon detection.

In collaboration with the research groups of Protein Structure and Dynamics and Structural Biology of Gene Regulation, the users can in collaborative projects utilize the expertise of the groups in studies of the structures, dynamics and interactions of proteins and nucleic acids, including the use of available hardware and software.

5. Cryo-electron Microscopy and Tomography

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

The cryo-EM core facility provides access to high-end instrumentation that is set up for high-throughput acquisition of cryoEM micrographs for single particle analysis and high-resolution 3D image reconstructions as well as for automated acquisition of cellular cryo-electron tomograms. The centre will also provide assistance in advanced sample preparation techniques (cryo-FIB milling) and data/image processing methods.

Instruments available:

- FEI Titan Krios – the microscope operates at 300 kV and is equipped with an autoloader, FEI Falcon II direct detector, Gatan Quantum 964 energy filter and a post-GIF 4k CCD camera. The microscope is optimally suitable for high-resolution single particle cryo-electron microscopy and cellular cryo-electron tomography.
- FEI Tecnai F20 – the microscope operates at 120–200 kV and is equipped with an FEI Eagle 4k CCD camera, two side-entry cryo-holders (Gatan 626 and Gatan 914) and two room-temperature side-entry holders. The microscope is optimally equipped for optimization of cryoEM samples and automated collection of data for initial 3-D reconstructions by single particle analysis.
- FEI Versa3D – this small dual beam microscope (SEM/FIB) is equipped with a Quorum cryo-stage for insertion and processing of cryo-TEM grids (imaging by SEM and lamellae milling with FIB). The samples can be Pt-coated inside the microscope.

Services provided:

The cryo-EM core facility provides access and support to collect cryo-electron microscopy images for both single particle as well as for electron tomography applications. Furthermore, the facility provides support for sample preparation, namely plunge freezing of purified protein complexes as well as cryo-FIB lamella milling for thick biological objects such as cells. Additional support is given in experiment design, setting up data collection and data analysis (i.e. image processing). Interested non-specialists could receive training in using the electron microscopes or develop a collaboration with the CryoEM research group, particularly in these areas:

- high-resolution structure determination of macromolecular complexes and assemblies
- structural studies of intracellular compartments and host-pathogen interactions
- time-resolved electron microscopy of transient macromolecular complexes

6. Proteomics Core Facility

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

Instruments available:

- IEF in solution - OFFGEL unit
- 1D and 2D gel electrophoresis - all necessary equipment including multi- gel units
- LC system for protein/peptide fractionation - Ultimate 3000 with Probot fractionator
- LC-MS/MS I - Ultimate RSLCnano + HCTUltra ion trap mass spectrometer with ETD
- LC-MS/MS II - Ultimate RSLCnano + Orbitrap Elite hybrid mass spectrometer with ETD
- LC-MS/MS III - Ultimate RSLCnano + Impact II Qq-Time-Of-Flight mass spectrometer
- LC-MS/MS IV - nanoLC system Eksigent + Qtrap6500 hybrid mass spectrometer for targeted proteomics

Services provided:

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

7. Biophysical methods

Core Facility contact: **Tatsiana Charnavets**, tatsiana.charnavets@ibt.cas.cz

Instruments available:

- Applied Photophysics circular dichroism (CD) spectrometer Chirascan Plus;
- UV/visible spectrophotometer with temperature control Analytic Jena Specord 50 Plus;
- BioRad ProteOn XPR36 interaction array instrument;
- Monolith NT.150 microscale thermophoresis (MST);
- Monolith NT. LabelFree thermophoresis;
- Prometheus NT.48 DSF assay;
- Microcal iTC200 highly sensitive, low volume isothermal titration calorimeter;
- Microcal VP-DSC highly sensitive differential scanning calorimeter;
- Malvern Zetasizer Nano ZS90.

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)

- 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 – 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.

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 (10^3 - 10^9 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.

Sumple 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.

8. Crystallisation of proteins and nucleic acids

Core Facility contact: **Jiří Pavlíček**, jiri.pavlicek@ibt.cas.cz

Instruments available:

- Spectrolight 600 - in drop dynamic light scattering measurements in Terasaki 72-well plates;
- ArtRobbins Gryphon dropsetter - a multi-channel (96 channels) pipetting robot for the easy set-up of nanodrop crystallisation plates;
- Formulatrix RI1000 crystallisation hotel - a crystallisation plate storage and automated crystallization monitoring enclosure allowing remote access to crystallization images. Crystallisation drop images can be taken using visible light, polarized light and UV;
- Glovebox with stereomicroscope for crystallization and crystal manipulation under defined (oxygen-free) atmosphere.

9. X-ray structure determination and analysis

Core Facility contact: **Karla Fejfarová**, karla.fejfarova@ibt.cas.cz
Jiří Pavlíček, jiri.pavlicek@ibt.cas.cz
Frederic Vellieux, frederic.vellieux@ibt.cas.cz

Instruments available:

- Bruker D8 Venture diffractometer with a high-flux liquid Gallium X-ray source MetalJet D2, Photon II detector and Kappa goniometer. The diffractometer is used (at CMS) for X-ray diffraction of biomolecular crystals.
- ISX stage for D8 Venture – motorized stage for in-situ X-ray diffraction experiments, enabling screening of diffraction properties in crystallization trays.

Services provided within the Core facility Crystallisation of proteins and nucleic acids, and X-ray structure determination and analysis:

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)

- Robotic setup of 96-well crystallisation plates, for screening of crystallisation conditions (also for “routine crystal production”) - for proteins, nucleic acids, complexes of biological macromolecules;
- Manual setup of crystallisation plates, for screening, crystal optimization or “routine crystal production”, using different plate formats. Crystallisation can be setup in a cold room, at room temperature (20°C) or in a warm room (26°C), all rooms being equipped with dedicated binoculars for plate setup and inspection;
- Idem, under an inert atmosphere in a glove box (20°C);
- Automated monitoring of crystallisation in the Formulatrix crystal hotel. Lighting using visible light, polarized light and UV light. Remote access to crystallisation droplet images, and automated preliminary evaluation;
- Crystal handling and preparation for diffraction experiments, including crystal fishing from droplets, cryo-protection, cryo-cooling (vitrification in liquid N2) and possibly long term storage in specialized Dewars containing liquid N2;
- Idem, either in oxygen-free conditions or with the preparation of Xe derivatives, also possibly with long-term storage in specialized Dewars;
- Supply of mounted cryo-loops;
- In-situ (in the crystallisation plates) testing of crystal diffraction using the ISX stage;
- Testing of diffraction using mounted crystals and / or measurement of X-ray diffraction data;
- Diffraction data processing, providing a data file (such as an MTZ file);
- Assistance / advice to solve a 3D structure (including a full 3D structure determination service on request);
- Measurement of X-ray diffraction data sets at synchrotron radiation sources.

10. Advanced Mass Spectrometry

Core Facility contact: **Petr Pompach**, petr.pompach@biomed.cas.cz

Instruments available:

- Bruker Daltonics 15T-Solarix XR FT-ICR mass spectrometer, with electrospray and MALDI ion sources. This ultra-high resolution mass spectrometer is used mostly for the determination of the precise mass of biological macromolecules, and the characterization of their posttranslational modifications. Further possibilities include peptide mass fingerprinting, detection of small molecule/metabolites, monitoring of protein structural changes and protein-protein interactions under physiological conditions by hydrogen-deuterium exchange, chemical cross-linking and covalent labelling;
- Agilent Technologies 1200 HPLC system (usually coupled to the 15T-SolariX XR mass spectrometer) for the separation of complex peptide mixtures, proteins and metabolites.

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 FT-ICR mass spectrometer with sequence confirmation by Top-down approach using different fragmentation techniques (collision induced dissociation, electron transfer/capture dissociation);
  - Peptide mass fingerprinting – identification of proteins from gel or solution including larger protein mixtures by using MALDI or ESI ;
  - Characterization of posttranslational modification such as phosphorylation, glycosylation or disulphide bonds ;
  - Structural mass spectrometry: limited proteolysis, hydrogen/deuterium exchange, chemical cross-linking, covalent labelling;
  - HPLC separation of peptides, proteins and small molecules (metabolites) coupled with mass spectrometric detection by FT-ICR;
  - Processing and interpretation of mass spectrometric data.