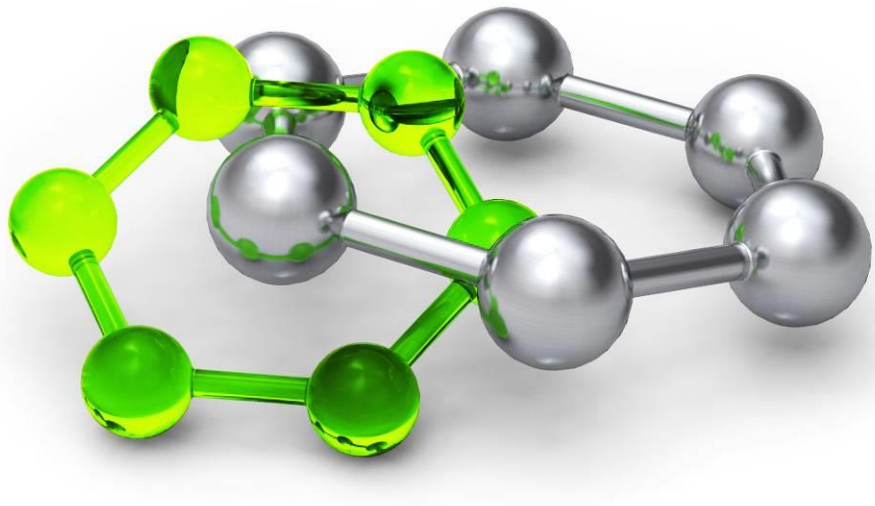
CEITEC

Central European Institute of Technology

BRNO | CZECH REPUBLIC

# **Equipment and services** of CEITEC core facilities available for **2015 call** for proposals

Updated on 7th April 2015



CEITEC

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

2007–13
OP Research and Development for Innovation

# Content

# 1. Nanofabrication and Nanocharacterization

Core Facility contact: **David Škoda**, david.skoda@ceitec.vutbr.cz

Core facility Nanofabrication and Nanocharacterization covers the unique technology portfolio and expertise in the field of e.g. top-down and botom-up approach in the fabrication of 0D – 2D nanostructures (metallic, semiconducting, high-K dieletric, non-conductive), surface process studies (controlled selective growths of nanostructures, Ga and Ge nanowires, graphene based materials) with application in plasmonics, nanomagnetism or optoelectronics.

Core facility provides the unique complete set of methods for nanofabrication and nanocharacterization and carry out both complete fabrication process of nanodevices, and their characterization of down to the sub-nanometre level in a clean environment (ranging from the class 100 to 100 000). All equipment for nanofabrication and most of the equipment for nanocharacterization is concentrated within a single cleanroom that decreases the risk of sample contamination and increases effectiveness of research.

The Core facility is a part of the research infrastructure CEITEC Nano.

**Available instruments and techniques within the project CEITEC open access:**

**Nanolithography infrastructure:**

- Optical Microscope – Olympus MX 51 – up to 6 inch sample size, from 5x to 100x magnification, bright and dark field option, Nomarski contrast, digital camera, sample inspection
- Mechanical profilometer - Bruker Dektak XT - mechanical profilometer, measurement of layer thickness
- Spincoater – Laurel 400 – up to 4 inch size, programmable, resist coating
- Centrifuge – up to 100 ml volume, resist cleaning
- Spectroscopic Reflectometer – Ocean Optics NanoCalc 2000, up to 6 inch sample size, visible to near infrared spectral range, 200 µm spot size, thin multilayer sample inspection – refractive index, thickness
- UV Direct Write Laser system - Heidelberg Instruments DWL 66 FS - UV lithography, mask preparation, custom design lithography up to 8 inch sample size
- Wetbench – chemical wet etching and cleaning, lithographic process development, hot plates, DI water, air conditioning
- Scanning Electron Microscope/e-beam writer – Tescan Mira3 with an interferometric high resolution lithographic table – up to 4 inch sample size, 200 V - 30 kV, electron beam lithography process
- Mask Photolithography – Perkin Elmer – 4 inch mask system, positive lithography process, 3 µm linewidth

**Chemical and thermal processes:**

- QDs synthesis – synthesis of quantum dots from the liquid solution
- MNPs synthesis – synthesis of magnetic nanoparticles from the liquid solution
- Surface nanostructuring – anodic oxidation for nanocolumns and nanopores fabrication, galvanic plating for nanocolumns and nanowires fabrication

**Etching & Deposition:**

- Atomic Layer Deposition system – Cambridge NanoTech Fiji 200, up to 8" sample size, capability to deposit thin films of $Al_2O_3$, $TiO_2$, TiN, AlN, MgO, $HfO_2$, $La_2O_3$, $Nb_2O_5$, $Ta_2O_5$, thermal and plasma RF mode of operation, process temperature range up to 400 °C
- Resist stripper – Diener NANO Plasma cleaner, stripping the resist by O2 and activation of surfaces by O2 microwave plasma
- Ion Beam Assisted Deposition – up to 0.5 inch size, HV environment, Au, Fe, Al, $Al_2O_3$, invar, Cu, Ti, Co, permalloy, $TiO_2$, TiN programmable controlled deposition
- Evaporation – up to 0.5 inch size, UHV environment, ultrathin films of Ga, Ge, Si, Cu, Fe, Ag, Au, Co
- Effusion cell – thermal hydrogen atom-beam source, up to 0.5 inch size, UHV environment
- Thermal evaporation – Balzers – thin layer deposition, liquid metal condensation principle

**Packaging & Testing:**

- Wire Bonder – TPT HB 16 wire bonder with two motorized axis, wedge bond process, heating stage, Au wire, micro/macro electrodes contacting

**Optical measurements:**

- NIR/VIS/UV (VUV) Spectroscopic Ellipsometers – J. A. Woollam IR-Vase, V-Vase – up to 6 inch sample size, visible to middle infrared spectral range (2 instruments), fully automated, sample optical characterisation
- Scanning Probe Microscope + microRAMAN + PhotoLuminiscence system – NT-MDT NTegra Spectra, Solaris 3 - system combined with a scanning confocal microRaman spectrometer – independent SPM and microRaman units, reflection mode, UV+VIS+NIR lasers, white-light laser supercontinuum source <420 nm; 2000 nm>, TERS option, information on topography and chemical composition, and photoluminescence properties of samples
- Vacuum FTIR – Bruker Vertex 80 + Hyperion 3000 – vacuum transmission reflection system, near to middle infrared spectral range, array detector, sample optical characterisation
- Photoluminescence – up to 1 inch sample size, non-destructive optical measurement of samples (information about the electronic structure, crystallinity and purity of semiconductors)
- Optical spectroscopy - up to 1 inch sample size, optical characterization of samples (dielectric properties, refractive index), from UV to FIR, reflectance, transmittance

- microRaman spectroscopy – Renishaw In-Via – microRaman spectroscopy, red and green laser excitation, spectroscopic technique, study of vibrational, rotational, and other low-frequency modes in a system (chemical composition information)
- Magneto-optical Kerr effect measurement - up to 0.5 inch sample, magnetic properties of materials (incl. magnetic anisotropy)
- VUV spectrometer VUVAS 1000

**Probe microscopy and Nanomanipulation:**

- Scanning Near-field Optical Microscope – NTegra Solaris, fiber SPM tip systems, transmission/reflection with illumination/collection SNOM modes, PMT detectors, information about the sample topography and near-field light interaction
- Scanning Near-field Optical Microscope – NTegra Spectra, aperture SPM tip systems, reflection with illumination/collection SNOM modes, PMT detectors, information about the sample topography and near-field light interaction
- Scanning Near-field Optical Microscope – Nanonics MultiView 4000, fiber SPM tip systems, transmission/reflection with illumination/collection SNOM modes, PMT and APD detectors, information about the sample topography and near-field light interaction
- Focused Ion Beam/Scanning Electron Microscope with nanomanipulators - Tescan Lyra3 XMH, system of 4 independent nanomanipulators combined with scanning electron and ion microscopy (SEM + EBL, FIB), up to 4 inch sample, Energy Dispersive X-ray Spectroscopy analysis, electric (4 probe) measurement, sample modification
- X-ray reflection – Huber, Digital Instrument – up to 1 inch sample size, study of crystallinity
- Coherence Controlled Holographic Microscope – CCHM - transmitted-light system adapted for live-cell time-lapse observations (heated box, flow chambers, anti-vibration system), reflected-light system, image-processing SW for on-line holographic reconstruction and evaluation of quantitative phase contrast
- Confocal Microscope (inverted transmission) - Nikon A1R - confocal fluorescence, reflection interference contrast, transmission mode
- Cell cultivation instruments – Schoeller, Trigon Plus, Nikon - laboratory equipment for the cell growth: incubator, flow-box, phase contrast imaging
- Microtomographic station "v|tome|x L 240" from "GE Phoenix" - accelerating voltage up to 240kV, sample size 1-500 mm, max 50 kg weight
- Metrology SPM – Nanopositioning and Nanomeasuring system (NMM1, Sios) for SPM, optical and tactile measurements at nanometer resolution up to the centimetre range.
- Focused Ion Beam/Scanning Electron Microscope DualBeam™ - FEI Quanta 3D 200i
- Lithographic Scanning Probe Microscope – Dimension Icon - scanning probe microscopy for surface studies - morphology, local electric and magnetic forces, conductivity, additionally lithography performed by local anodic oxidation, up to 8 inch sample size

**Electrical and Magnetic measurements:**

- Semiconductor measurements – Keithley 4200 - V-A, C-V, resistance characteristics

- Low Temperature Electro-Magnetic Properties Measurement System - Lake Shore CRX-EM-HF - 4-probe station operating in extended temperature range combined with horizontal magnetic field

**In-situ Fabrication / Analysis:**

- Secondary Ion Mass Spectroscopy – up to 0.5 inch UHV compatible sample, surface sensitive method, destructive elemental analysis of samples based on ion-beam sputtering and secondary ion detection, depth resolution- tens of nanometres, smallest inspected area below 1 $cm^2$
- X-ray Photoelectron Spectroscopy – up to 0.5 inch UHV compatible sample, surface sensitive method, non-destructive chemical analysis of samples based on X-ray radiation and photoelectron detection, Mg, Al anode, smallest inspected area below 1 $mm^2$
- Thermal Desorption Spectroscopy – up to 0.5 inch UHV compatible sample, surface sensitive method, destructive elemental analysis of samples based on sample heating and residual gas mass analysis
- Low Energy Electron Diffraction – up to 0.5 inch UHV compatible sample, surface sensitive method, non-destructive analysis of surface atomic structure based on low energy electron bombardment of sample surfaces and their diffraction
- Reflected High Energy Electron Diffraction (RHEED) – up to 0.5 inch sample size UHV compatible, surface sensitive method, non-destructive analysis of surface atomic structure based on high energy electron bombardment and their diffraction
- Low Energy Ion Scattering – up to 0.5 inch UHV compatible sample, surface sensitive method, elemental surface composition analysis based on interaction between incoming low energy ions and surface particles

**Services provided:**

- Lithography processes for nanostructure fabrication – preparation and modification of nanostructures, and patterning the samples by processes of
    - UV direct write lithography – custom pattern, resolution 1 µm, up to 8” sample size
    - electron beam lithography – custom pattern, resolution 10 nm, up to 4” sample size
    - ion beam lithography – custom pattern, resolution 20 nm, up to 4” sample size, Ga+ ions
    - lithography by atomic force microscopy - anodic oxidation, custom pattern, up to 8” sample size, resolution 100 nm
- Thickness and optical constant (index of refraction and absorption) characterization of thin multilayer systems using spectroscopic reflectometry – up to 8” sample size, active area 200 µm2
- Large (8”) and small (<0.1 nm) scale up to nanometer level of thickness and profile analysis of surfaces using mechanical profilometry and scanning probe microscopy – up to 8” sample size
- Large (8”) scale stress analysis using mechanical profilometry

- Surface etching of organic resists and activation of surfaces by microwave oxygen plasma – up to 8” sample size using resist stripper
- Monoatomic surface deposition of high-K dielectric layers based on oxides and nitrides - up to 8” sample size using atomic layer deposition system
- Electrical wiring of samples for testing and measurement – from small area (0.05”) contact pads using wire bonder
- Measurement of optical properties as a refractive index and dielectric function, absorption, emission, resonant frequencies, scattering and coherency of atoms, molecules – combination of local and area techniques, up to 4” sample size using spectroscopic ellipsometry and spectrometry
- Surface modification of local areas (up to 0.05”) as a fine and rough milling with resolution < 10 nm, etching (H20, F) and deposition (W, Co, Pt) with resolution < 50 nm using combination of scanning electron microscopy with focused ion beam and gas injection system technology – up to 4” sample size,
- Detailed surface visualization of structure with true 3D resolution using the scanning probe microscopy and scanning electron microscopy (2D) – up to 8” sample size, resolution < 10 nm
- Mapping the near-field light interaction and distribution of transmitted and reflected incident light – up to 0.5” sample size, resolution < 100 nm, active area up to 70 x 70 µm2 using two independent probe scanning near-field optical microscopy
- Mapping the electrical and magnetic force distribution and electrical work function of the sample surface – up to 8” wafer size using advanced techniques of atomic force and scanning tunneling microscopy, resolution < 100 nm, active area up to 0.05”
- Establishing the local/area surface chemistry using optical methods as FT-IR, Raman spectroscopy – up to 4” inch sample size, resolution 10 µm, active area < 0.1 nm; and energy dispersion X-ray spectroscopy - – up to 4” inch sample size, resolution 1 µm, active area 100 µm

The Core Facility Staff provides the users by training in operation of instruments, support in experiments, and helps with data analysis.

# 2. Structural Analysis Laboratory

Core Facility contact: **Ondřej Man**, ondrej.man@ceitec.vutbr.cz

The core facility will be - after completion - equipped with top-class instruments for transmission and scanning electron microscopy, microanalysis and X-ray diffraction. There will also be sufficient background for preparation of samples from various materials that can be described as “non-life science”. The people within the core facility participate in various research activities which have a close relation to the activities of the Advanced Ceramic Materials, Advanced Polymers and Composites, and Cybernetics in Material Science research groups. The priority is to focus on the study of the microstructure, submicrostructure and local chemical analysis of new advanced ceramic and polymer materials and composites based on those materials. Another research area is the nanocrystalline thermal barrier coatings and materials with ultra-fine grains obtained via SPD (Severe Plastic Deformation).

In its fully operational state, the core facility will provide high-level instruments such as HR TEM, HR SEM, FIB/SEM, X-ray diffractometer with high brightness source and an SAXS -enabled X-ray diffractometer. In the background, there will be fully equipped laboratories dedicated to sample preparation.

**Available instruments and techniques:**

- X-ray Powder Diffractometer Rigaku SmartLab 3kW with attachments (high temperature chamber up to 1600°C, low temperature chamber down to -190°C and high temperature reactive chamber up to 900°C). Line focussed X-ray source (Cu, Co as standard) and variable X-ray optics in primary and scattered beam path. Equipped with various environmental chambers (heated, cooled, vacuum and gas flow chamber) and carousel for rapid specimen exchange. Phase identification, quantification, residual stress measurement, thin layer thickness determination.
- X-ray Diffractometer (with rotating Cu anode) for thin films measurements, Rigaku SmartLab 9kW with attachments (high temperature C-dome chamber up to 1100°C, software for qualitative and quantitative analysis, crystallite size and lattice strain analysis, texture and stress analysis, etc.). High brightness Cu X-ray source and swivelling detector arm allow reciprocal space mapping in wider range. Able to accept various environmental chambers.

**Services provided:**

- Phase Identification and Structure analysis (quantitative and qualitative analysis, lattice constants and crystal structure refinement from measurement of sample in Bragg-Brentano (BB) geometry; precious analysis in Parallel Beam (PB) geometry)

- Film thickness measurement – X-ray reflectivity (roughness, density and thickness of layers; medium resolution in PB, high and ultra high resolution 2- and 4- bounce germanium monochromator)
- Crystal quality analysis – rocking curve and reciprocal space mapping, evaluated crystallinity of thin film formed on substrate (medium resolution in PB, high and ultra high resolution with 2- and 4- bounce monochromator)
- Texture measurement – pole figure and in-plane pole figures (preferred orientation of sample)
- Nano-structure analysis – small angle x-ray scattering (SAXS) and ultra small angle x-ray scattering (USAXS) in transmission and reflection mode (size distribution of particles, pores, thin films and superlattice; 1nm-1μm)
- Residual Stress measurement - (40 x 40 x 10 mm sample)
- Microdiffraction and mapping – X-ray beam focused down to area of 0.5x0.5 mm
- In-situ measurements - high temperature chamber up to 1600°C (vacuum, inert gases; powder and thin samples), low temperature chamber down to -196°C (small bulk samples), high temperature reactive chamber up to 900°C (reactive atmosphere; small bulk samples)

The Core Facility Staff offers the users a support for the experiments, and helps with data analysis, provides the training courses for chosen instruments.

# 3. 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.cz
- Calorimetry: eva.dubska@ceitec.cz
- SPR: lenka.malinovska@ceitec.cz
- AUC: jan.komarek@ceitec.cz
- Crystallization and structural studies: josef.houser@ceitec.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.

*Core facility Biomolecular Interactions and Crystallization is part of Czech National Affiliated Centre of Instruct.*

**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, $K_a$, $\Delta H$ and $\Delta S$ 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 ($\Delta C_p$) 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.

**Sample requirements - importance of sample preparation**

- 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.

**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.

# 4. X-ray Diffraction and Bio-SAXS Core Facility

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

*X-ray Diffraction and Bio-SAXS Core Facility is part of Czech National Affiliated Centre of Instruct.*

**Instruments available:**

- Robotized macromolecular diffraction system Rigaku HighFlux HomeLab™ with sample changer ACTOR optimized for work at Cu-$K_\alpha$ wavelength
- Universal, dual wavelength (Mo-$K_\alpha$ and Cu-$K_\alpha$) 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

# 5. Nanobiotechnology Core Facility

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

*Nanobiotechnology Core Facility is part of Czech National Affiliated Centre of Instruct.*

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.

# 6. 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.

*Core facility Josef Dadok National NMR Centre is part of Czech National Affiliated Centre of Instruct.*

**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.

# 7. Cryo-electron Microscopy and Tomography

Core facility contact: **Daniel Němeček**, daniel.nemecek@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.

*Core facility Cryo-electron Microscopy and tomography is part of Czech National Affiliated Centre of Instruct.*

**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

# 8. Proteomics Core Facility

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

*Proteomics Core Facility is part of Czech National Affiliated Centre of Instruct.*

**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

# 9. Genomics Core Facility

Core Facility contact: **Boris Tichý**, boris.tichy@ceitec.muni.cz

**Instruments available:**

- Agilent SureScan Microarray Scanner
- Roche GS Junior – benchtop massively parallel sequencer
- Illumina MiSeq – benchtop massively parallel sequencer
- Illumina NextSeq – whole-genome massively parallel sequencer
- Life Technologies 3500 Genetic Analyzer – capillary sequencer
- Life Technologies QuantStudio 12k – high-throughput quantitative and digital PCR
- Life Technologies QuantStudio 3D – digital PCR
- Wafergen SmartChip – high-throughput qPCR and NGS target enrichment
- Fluidigm AccessArray – NGS target enrichment

**Software available:**

- Agilent GeneSpring GX – microarray analysis
- Agilent Genomic Workbench – microarray analysis
- CLCBio Genomics Workbech – sequence analysis

**Services provided:**

- Consultation
- Microarray – gene expression and CGH arrays
- Sequencing – capillary, MiSeq, NextSeq, 454
- NGS library preparation

# 10. MAFIL – Multimodal and Functional Imaging Laboratory

**Core Facility contacts:**
Michal Mikl, michal.mikl@ceitec.muni.cz – deputy head, CF manager
Lubomír Vojtíšek, lubomir.vojtisek@ceitec.muni.cz – technical support and reservations for MRI
Martin Kojan, martin.kojan@ceitec.muni.cz – technical support and reservations for EEG

The core facility provides technologies and methods for in-vivo magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) with spatial resolution reaching to 0.25 mm, with the main application in functional (fMRI), multimodal, and multiparametric imaging of the brain. There is also possibility of high density electroencephalography (HD-EEG) recordings during MR experiment or in separate RF-shielded laboratory.

**Services are related to these topics:**

- advanced structural in-vivo or ex-vivo imaging at high resolution
- MRS and magnetic resonance spectroscopic imaging (MRSI)
- fMRI of brain including anatomical and functional connectivity studies
- dual fMRI experiments (simultaneous measurements with 2 scanners)
- multimodal and multiparametric imaging (e.g. fMRI+EEG/EP, fMRI+DTI)
- designing fMRI and connectivity studies
- advanced MRI, fMRI, MRS multimodal data processing
- high density EEG (256 channels) recordings

**Equipment:**

- Two whole-body human 3T MR scanners Siemens Prisma (gradient system with amplitude 80mT/m and slew-rate 200 mT/m/s; excitation with two independent channels; 64 channel head-neck coil, 20 channel head-neck coil; 32 channel spine coil, 18 channel body coil, small and large 4 channel flexible coils)
- MR compatible high density EEG system EGI Net Amps 400 (256 channels)
- MR compatible EEG/ExG system Brain Products MR (30 EEG channels, ECG, EOG, skin conductance, EMG, breathing, acceleration, general bipolar measurements)
- MR compatible ExG (polygraph) system Brain Products MR (ECG, skin conductance, EMG, breathing, acceleration, general bipolar measurements)
- MR compatible stimulation system for both MR scanners
- RF-shielded laboratory

*CEITEC – open access project, ID number LM2011020, is funded by the Ministry of Education, Youth and Sports of the Czech Republic under the activity „Projects of major infrastructures for research, development and innovations".*