



CEITEC

Central European Institute of Technology
BRNO | CZECH REPUBLIC

Josef Houser

Core Facility Head

6. 9. 2023,
Summer Workshop on BioAFM Microscopy 2023

CF BIC Biomolecular Interactions and Crystallography

MUNI



Biomolecular Interactions and Crystallography CF

- Part of CEITEC MU Core Facilities
- Established in 2014
- 16 techniques with 30 instruments



Biophysical characterization

- Homogeneity
 - Molecular mass
 - Oligomeric state
 - Aggregation
- Stability
 - Temperature
 - Buffer composition
- Folding
 - Secondary structure
 - Shape

- Analytical ultracentrifugation (AUC)
- Size exclusion chromatography (SEC-LS)
- Dynamic light scattering (DLS)
- Differential scanning fluorimetry (DSF)
- Differential scanning calorimetry (DSC)
- Circular dichroism spectroscopy (CD)
- UV/VIS spectroscopy
- Small-angle X-ray scattering (SAXS)



Optima AUC



ProteomeLab XL-I



Jasco J-815



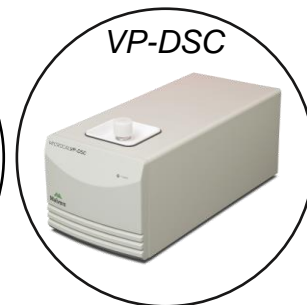
Auto PEAQ-DSC



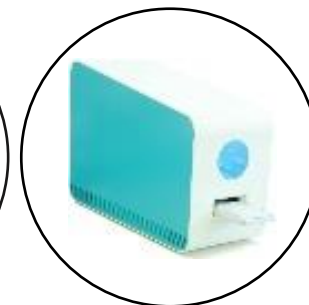
DelsaMAX



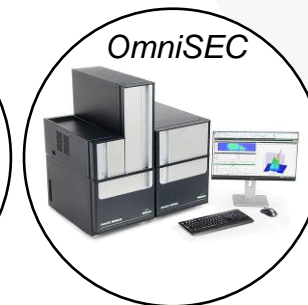
Prometheus NT.48



VP-DSC



Spectrolight 600



OmniSEC



BioSpectrometer kinetic



BioSAXS 2000

Biomolecular interactions

- Various principles

- In solution
- On surface
- Cell-based

- Parameters

- Affinity
- Thermodynamics
- Kinetics

- Types of molecules

- Proteins, nucleic acids, small molecules

- Isothermal titration calorimetry (ITC)

- Micro-scale thermophoresis (MST)

- Bio-layer interferometry (BLI)

- Surface plasmon resonance (SPR)

- Analytical ultracentrifugation (AUC)

- Flow cytometry with sorting (FC)



Optima AUC



ProteomeLab XL-I



Multichannel SPR



Monolith NT.117



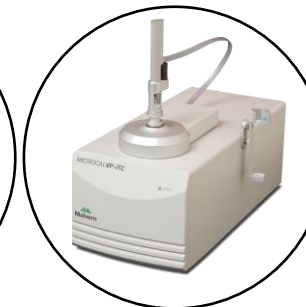
Auto PEAQ-ITC



Biacore S200



Octet RED96e



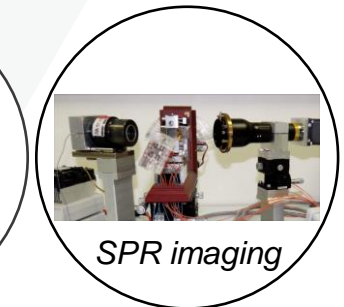
VP-ITC



Monolith NT.117 Pico



CytoFLEX



SPR imaging

Crystallography

- Complete instrumentation for crystallization screening
- Optimization of crystallization
- Diffraction data collection
- 3D structure determination

- Crystallization plates set-up robotics
- Crystal plates storage and imaging
- Crystallization temperature optimization
- Optical microscopes
- X-ray diffraction
- Accessories for cryo-crystallography



Phoenix



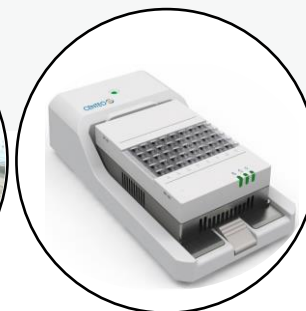
Mosquito



SpectroLight (20°C)



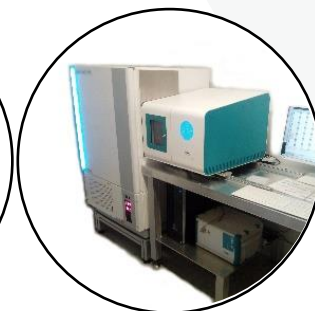
TG40



epMotion



SpectroLight (4°C)



SMZ800



High throughput

- Robotization and automation
- Pipetting from 300 pl to 1 ml
- Microarrays, 96 and 384 plates handling

- **Parallelization**
 - In plates – DLS, BLI, crystallization
 - In capillaries – DSF
- **Autosamplers**
 - Walk-away operation, 24/7
 - SPR, ITC, DSC, SEC-LS
- **Pipetting robotics**
 - SBS plate preparation
 - Microarrays

sciFLEXARRAYER



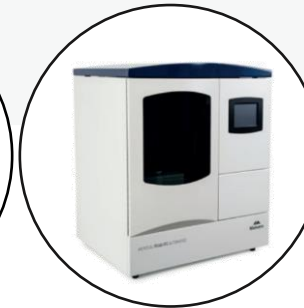
Phoenix



epMotion



Auto PEAQ-ITC



Biacore S200



CF BIC users

- 90 individual **users** from 40 **groups** per year
- 10 000 **machine hours** per year
- Internal, external, foreign users

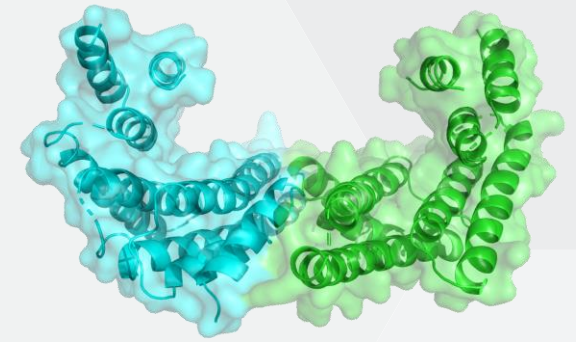


FI **BIOVIAN** **US** **Midwestern University** **BE** **KU LEUVEN**
PL **Poznań University of Life Sciences** **FR** **UNIVERSITY OF TRENTO**
MX **POLITÉCNICA DE SANTA ROSA** **HR** **University of Ljubljana** **SI**

Recent publications

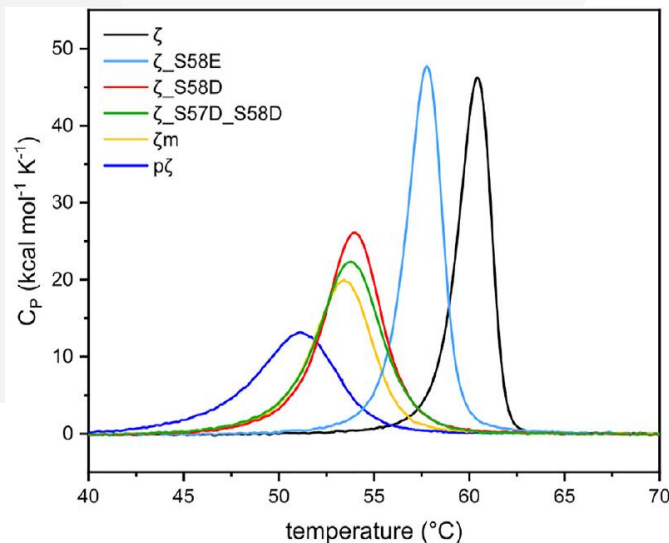
- **Simonic M. et al.** (2023) Biomolecular Complexation on the “Wrong Side”: A Case Study of the Influence of Salts and Sugars on the Interactions between Bovine Serum Albumin and Sodium Polystyrene Sulfonate. *BIOMACROMOLECULES* ,
- **Houser J. et al.** (2023) The NFκB activation domain is 14-amino-acid-long variant of the 9aaTAD. *BIOCHEM J*
- **Veverka P. et al.** (2023) Electron microscopy reveals toroidal shape of master neuronal cell differentiator REST - RE1-silencing transcription factor. *COMP STRUCT BIOTECH J*
- **Shukla S. et al.** (2022) In-solution structure and oligomerization of human histone deacetylase 6 - an integrative approach, *FEBS J*
- **Rando C. et al.** (2022) Highly Efficient and Selective Recognition of Dicyanoaurate(I) by a Bambusuril Macrocyclic in Water, *Angew. Chem. Int. Ed.*
- **Tharra P. R. et al.** (2022) Short Synthesis of (+)-Actinobolin: Simple Entry to Complex Small-Molecule Inhibitors of Protein Synthesis, *Angew. Chem. Int. Ed.*
- **Kasiliauskaite A. et al.** (2022) Cooperation between intrinsically disordered and ordered regions of Spt6 regulates nucleosome and Pol II CTD binding and nucleosome assembly, *Nucleic Acids Res.*
- **Bilanovicova V. et al.** (2022) The Hydrophilic Loop of Arabidopsis PIN1 Auxin Efflux Carrier Harbors Hallmarks of an Intrinsically Disordered Protein, *Int J Mol Sci*
- **Kozelekova A. et al.** (2022) Phosphorylated and phosphomimicking variants may differ - a case study of 14-3-3 protein, *Front Chem*
- **Trosanova Z. et al.** (2022) Quantitation of Human 14-3-3zeta Dimerization and the Effect of Phosphorylation on Dimer-monomer Equilibria, *J Mol Biol*
- *And other...*

Case study: 14-3-3 ζ dimerization

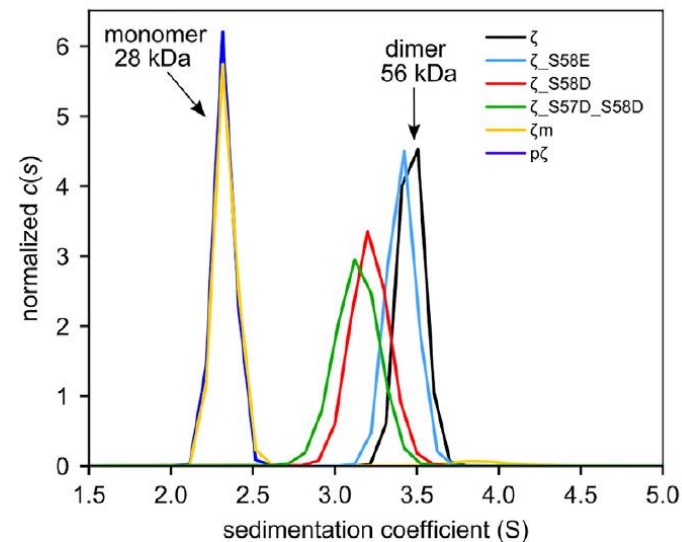


- Human **regulatory protein**
- Interaction with other proteins depends on dimerization which is affected by **phosphorylation**
- Study of phosphorylated protein and **phospho-mimicking** mutants revealed their non-equivalency

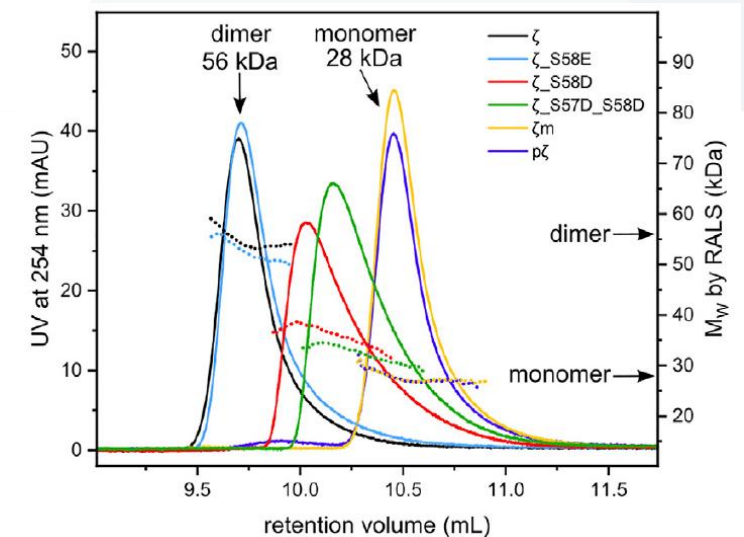
DSC



AUC

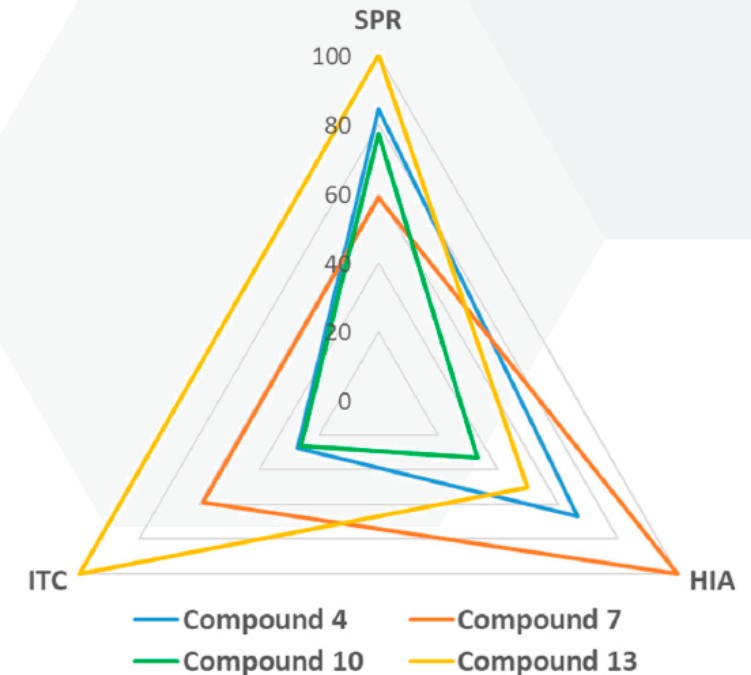
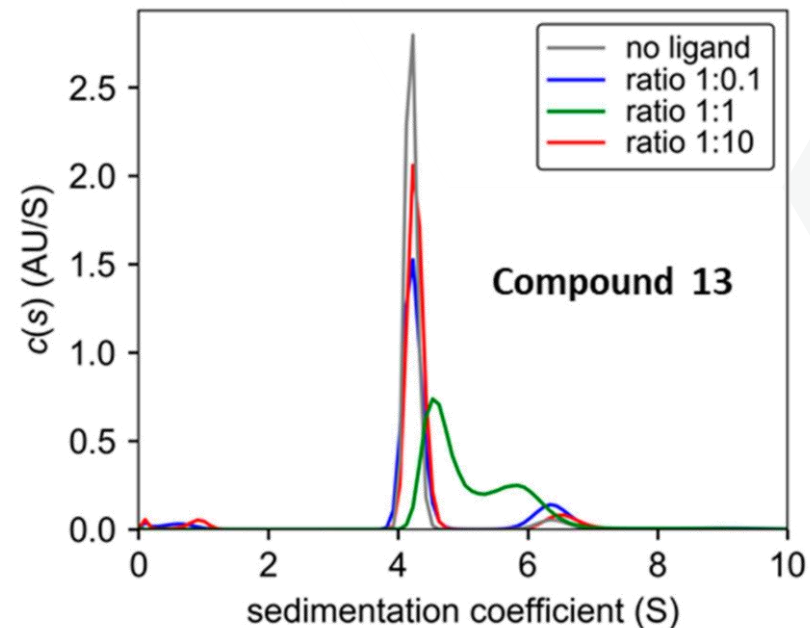
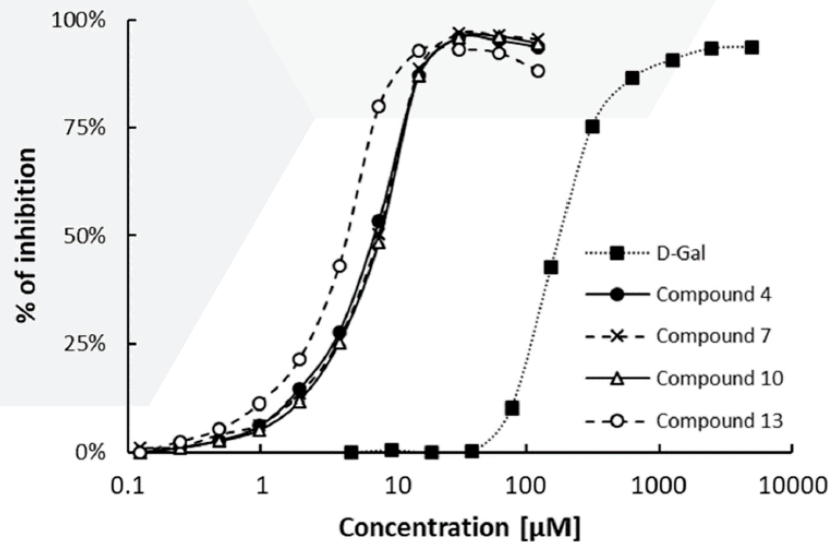
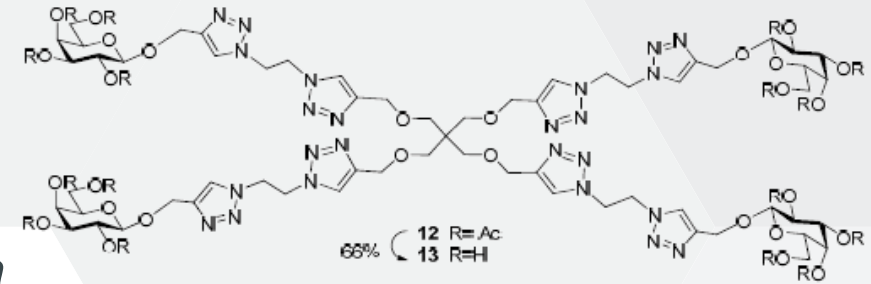


SEC-LS



Case study: Lectin inhibitors

- Lectin PA-IL from human pathogen *Pseudomonas aeruginosa*
- Development of inhibitors preventing pathogen invasion
- Several **multivalent inhibitors** tested on molecular and cell level
- **Promising compounds** recommended for in vivo testing



Access

- CF BIC is opened to everyone
- Majority of users from CEITEC and Masaryk University
- **Modes of access**
 - Full prices – based on cost matrix
 - **Reduced/no fee – national/international projects**
 - Collaboration – specific cases
- Compliance with **CF BIC rules** required
- **Acknowledgement** obligatory

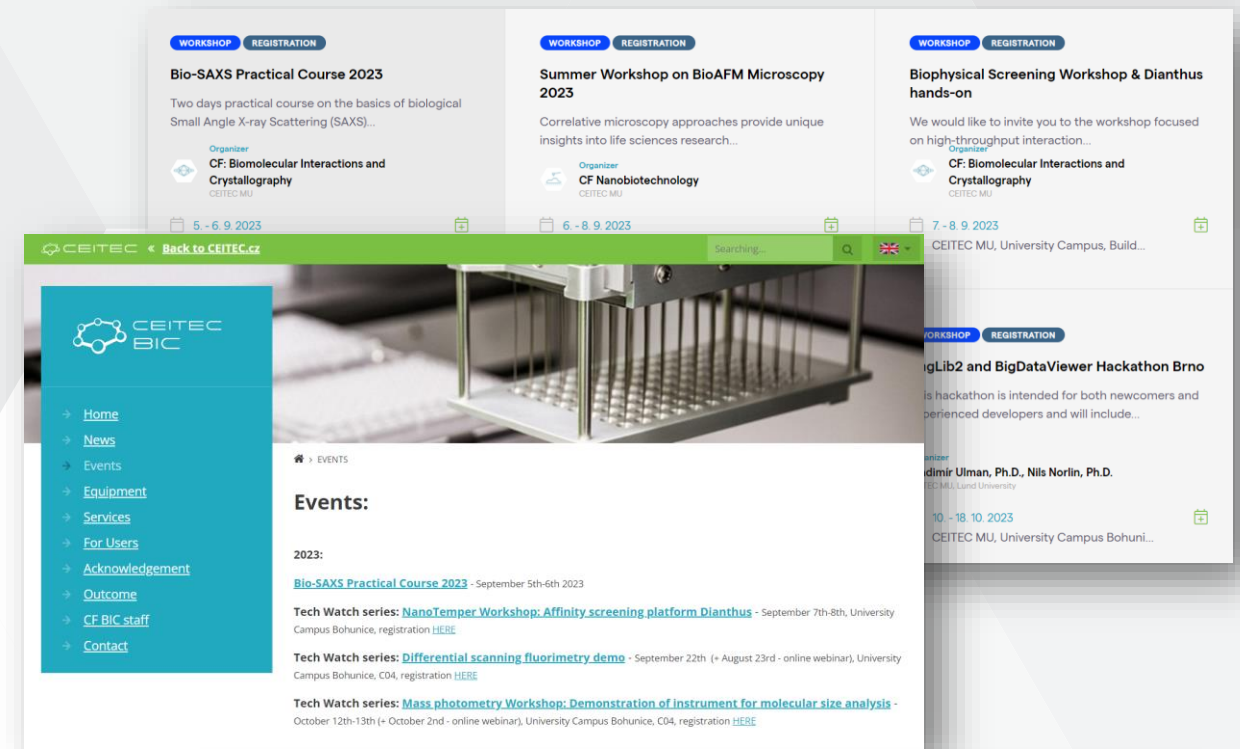


Alliance4Life



Education by Core Facility

- Various types of education
 - Individual trainings for instrument use
 - Group trainings
 - Data evaluation training
 - Dedicated workshops
 - Regular courses
- More details on
 - CF web page
 - CEITEC events
 - MUNI information system (IS)



S2004 Methods for characterization of biomolecular interactions

S1004 Methods for structural characterization of biomolecules

S5018 Methods of Protein Characterization

Faculty of Science
Spring 2023

▣ Extent and Intensity

1/0/0. 1 credit(s) (plus extra credits for completion). Recommended

▣ Teacher(s)

doc. RNDr. Radovan Fiala, CSc. (lecturer)
Mgr. Josef Houser, Ph.D. (lecturer)
Mgr. Pavel Kadeřávek, Ph.D. (lecturer)
Mgr. Tomáš Klumpler, Ph.D. (lecturer)
doc. Mgr. Karel Kubiček, PhD. (lecturer)
Mgr. Jiří Nováček, Ph.D. (lecturer)

Coming workshops

- **Biophysical screening** workshop

- High-throughput analysis of biomolecular interactions
- Practical hands-on demonstration of Dianthus and Panta instruments
- Discussion with experts
- Possibility to measure own samples

7 – 8 Sep 2023



- **Differential scanning fluorimetry** demo

- Analysis of temperature stability of proteins
- Practical hands-on demonstration of SUPR-DSF instrument
- Discussion with experts
- Possibility to measure own samples

22 Sep 2023



- **Mass photometry** workshop

- Demonstration of instrument for molecular size analysis
- Online webinar on method theory (2/10/2023)
- Discussion with experts
- Possibility to measure own samples

12 – 13 Oct 2023



Biomolecular
Interactions and
Crystallography



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Thank you for your attention.