



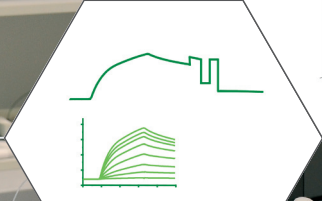
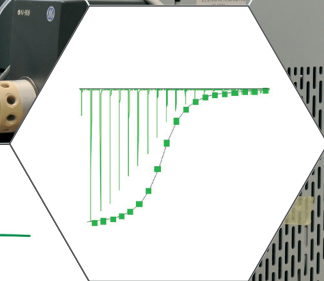
# Current State-of-Art to Study Biomolecular Interactions and Assemblies in Life Sciences

MONDAY

10/11/2014

Mendel Museum, Augustinian Abbey  
Mendlovo nám. 1a, Brno

## Programme of the workshop





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# PROGRAMME

Time	Lecture
09:00 - 09:25	registration
09:25 - 09:30	opening remark – <b>Michaela Wimmerová</b>
09:30 - 10:05	<b>Patrick England</b> A general overview of the world of biophysical approaches to study macromolecular assemblies and interactions
10:05 - 10:40	<b>Peter Gimeson</b> Managing heat and disorder, calorimetric assays in life sciences
10:40 - 11:10	Coffee break 
11:10 - 11:45	<b>Olwyn Byron</b> Biomolecular interactions observed and quantified by AUC and SAS
11:45 - 12:20	<b>Valérie Belle</b> Probing structural flexibility in proteins using site-directed spin labelling combined with EPR spectroscopy
12:20 - 13:50	Lunch 

<b>Time</b>	<b>Lecture</b>
13:50 - 14:25	<b>Arthur Sedivy</b> MicroScale Thermophoresis – a short history and outlook about the technique and its application
14:25 - 15:00	<b>Alexey Rak</b> The use of virtual screening, DSF and MicroScale Thermophoresis for the rapid identification of fragments active against MEK1
15:00 - 15:30	Coffee break 
15:30 - 16:05	<b>Gregor Anderluh</b> Interactions of pore-forming proteins with lipid membranes
16:05 - 16:40	<b>Frank Bernhard</b> Cell-free production of membrane proteins
16:40 - 16:50	closing remark



# PATRICK ENGLAND

Centre of Biophysics of Macromolecules and their Interactions, Institut Pasteur, Paris

## A general overview of the world of biophysical approaches to study macromolecular assemblies and interactions

Patrick England is the head of the Centre of Biophysics of Macromolecules and their Interactions (PFBMI), that he created in the Institut Pasteur (Paris, France) in 2002. This open core facility federates a large ensemble of state-of-the-art instrumentation for the in vitro molecular-scale characterization of biological macromolecules, together with well-established technical and methodological expertise, allowing to address questions concerning the study of intrinsic properties of macromolecules (e.g. shape, folding, stability, auto-association) and of the interactions in which they are involved (e.g. stoichiometry, thermodynamic and kinetic parameters). Before deciding to dedicate his efforts to the development of the PFBMI, Patrick England conducted

research on antibody engineering and on bacterial signal transduction.

In his introductory talk, Dr. England will give an overview of the very large variety of physico-chemical approaches that have been developed in the last decades to study the properties of macromolecular interactions. Among others, he will talk about isothermal titration calorimetry (ITC), surface plasmon resonance (SPR) and techniques apperanted to SPR (QCM, dual polarization interferometry, bilayer interferometry, back scattering interferometry).

He will also present the recently created Association of Resources for Biophysical Research in Europe (ARBRE), that already brings together 70 facilities from 15 european countries (see page 15).



# PETER GIMESON

Malvern Instruments Ltd.

## Managing heat and disorder, calorimetric assays in life sciences

Peter Gimeson joined Malvern Instruments Ltd. in 2014 in connection to the acquisition of MicroCal from GE Healthcare. Prior that, Peter worked at GE healthcare, Life sciences as senior application specialist for microcalorimetry covering Europe, Middle East and Africa. The function allowed him to interact with users on a daily basis regarding all aspects of instrumentation, assay developments and data analysis in Isothermal Titration Calorimetry and Differential Scanning Calorimetry.

assays in determining affinities and stability parameters in life science. It will highlight the added value given by thermodynamic profiling in order to differentiate interaction systems and monitor effects of modifications seen in enthalpy/entropy changes. High resolution thermal stability assay for domain de-convolution and how modifications and composition influences domain/global thermal stability will be also discussed. The lecture will cover instrumentation, application examples and sample preparation.

The lecture will discuss the use and application of calorimetric



# OLWYN BYRON

University of Glasgow, UK

## Biomolecular interactions observed and quantified by AUC and SAS

Olwyn Byron is a molecular biophysicist whose research is centered on meso-resolution solution structural biology, in particular the definition of solution macromolecular conformations and the characterisation of functional complexes in terms of strength and topology of interaction. This is achieved through the study of systems via methods such as analytical ultracentrifugation (AUC) and small-angle solution X-ray and neutron scattering (SAXS, SANS) and is of particular utility for systems that are refractory to crystallisation or NMR spectroscopy. The parameters so generated are interpreted using hydrodynamic bead modelling and *ab initio* molecular envelope reconstruction. Dr. Byron is currently developing SAUCE (Scattering-AUC Equipment): an ultracentrifugal sample environment that will facilitate the simultaneous acquisition of SAXS/SANS and hydrodynamic data for

complex for non-crystalline systems. She is also a member of SCAPA (the Scottish Centre for the Application of Plasma-based Accelerators), leading the development of SOLAS: a laser wakefield accelerator X-ray diffraction beamline with unique capabilities for the materials and life sciences.

In her lecture she will briefly present the principles underlying AUC, describe how AUC experiments are typically performed, the types of systems that can be analysed and the data that result. She will focus on the interpretation of these data using hydrodynamic modelling and will stress the synergy between SAS and AUC. The lecture will focus on examples of their use in effectively characterising the interactions between biomolecules. This will include examples of macromolecular interaction and also the interactions between proteins and small molecules.





# VALÉRIE BELLE

University of Marseille, France

## Probing structural flexibility in proteins using site-directed spin labeling combined with EPR spectroscopy

Valérie Belle is professor at the Aix-Marseille University and works in the laboratory of Bioenergetics and Protein Engineering. In the last few years she focused her research activities on the study of protein dynamics using Site Directed Spin Labeling combined with Electron Paramagnetic Resonance (SDSL-EPR) spectroscopy. She is interested in characterizing structural transitions within proteins mostly arising from protein-protein interaction, in particular in the family of intrinsically disordered proteins. She is also involved in the development of new paramagnetic spin labels aiming at enlarging the potentialities of SDSL-EPR.

In her talk she will present the principles of this approach going

from the classical technique using continuous wave EPR to the more advanced techniques based on pulsed EPR experiments. She will give examples showing how SDSL-EPR can successfully be applied to characterize the structural behavior of different biological systems. These examples will concern the conformational change of an interfacial enzyme, namely the Human Pancreatic Lipase, the flexibility of a chaperone dedicated to the assembly of a molybdoenzyme and the local disorder-to-order transition of viral nucleoproteins. Moreover she will present the recent development of new spin labels that enlarges the panoply of SDSL-EPR approaches by overcoming the limitations of the commercial labels.



# ARTHUR SEDIVY

CSF Protein Technologies Facility, Vienna, Austria

## MicroScale Thermophoresis – a short history and outlook about the technique and its application

Arthur Sedivy is an expert in various biophysical methods, in particular the recent method of Microscale Thermophoresis. In his current occupation at the Campus Science Support Facilities GmbH (CSF) in Vienna, he is providing services, including method training, experiment design and conduction of experiments, in various biophysical methods such as UV-Vis Spectroscopy, Circular Dichroism (CD), Microscale Thermophoresis (MST), Fluorescence Correlation Spectroscopy (FCS), Differential Scanning Calorimetry (DSC) and Differential Scanning Fluorimetry (DSF). He is interested in novel techniques as well as novel applications of established techniques

and their possible implementation in the sequence of experiments conducted by structural/molecular biologists.

Arthur Sedivy will give an introductory lecture on the technique of MST as well as show some examples of straightforward but also more advanced usage of the technique. The main part of the talk will focus on the invention of Microscale Thermophoresis and its general physical principle as well as how to measure biomolecular interactions. In addition, the advantages and disadvantages of MST over other available techniques, such as FCS, ITC and DSF will be discussed.



# ALEXEY RAK

Sanofi-Aventis R&D, Vitry, France

## The use of virtual screening, DSF and MicroScale Thermophoresis for the rapid identification of fragments active against MEK1

Alexey Rak is heading the structural biology and biophysics efforts at the LGCR (Lead Generation and Chemical Realisation) scientific platform of Sanofi R&D in France. During his career he has received several prestigious awards for his work in the field of vesicular membrane trafficking and small GTPases associated signaling. In 2007 he joined Sanofi, a leading pharmaceutical company based in Paris, France, and is now heading structural biology and biophysics based research in small molecules and biologics therapeutic projects for various human diseases indications at Sanofi R&D in France.

Biophysical approaches are routinely used to assess the binding constants of molecular interactions as integral part of the drug discovery process being essential for efficient lead optimization. Furthermore, modern drug discovery operations require characterization of biomolecular interactions to be both time- and cost-effective. Here we report the analysis of an in-house fragment screening campaign for the oncology target - MEK1. The application of

virtual screening (VS) as a primary fragment screening approach was followed by biophysical validation using Differential Screening Fluorimetry (DSF) and MicroScale Thermophoresis (MST), with resultant binding mode determination by X-ray crystallography. Using the Monolith NT.Automated instrument, we screened a library containing 193 pre-selected fragments for their interaction with MEK1. We identified >70 binders with dissociation constants (K<sub>d</sub>s) ranging from the low  $\mu\text{M}$  to low mM values. 16 fragments displayed K<sub>d</sub>s below 100  $\mu\text{M}$ . Importantly, 7 out of 8 previously determined hits were among the top-fifteen fragments from our MST-ranking and yielded x-ray co-crystal structures with MEK1. Moreover, the MST ranking showed a very strong correlation with a qualitative DSF screening. We demonstrate the effectiveness of the VS-DSF-MST workflow for the early identification of fragments to both 'jump-start' the drug discovery project and to complement biochemical screening data.



# GREGOR ANDERLUH

Infrastructural Centre for Molecular Interaction Analysis,  
University of Ljubljana, Slovenia

## Interactions of pore-forming proteins with lipid membranes

Gregor Anderluh is a head of the Laboratory for Molecular Biology and Nanobiotechnology at the National Institute of Chemistry, Slovenia, and Professor of Biochemistry at the University of Ljubljana, Slovenia. His main topics of research are protein-membrane interactions and, in particular, how cellular membranes are damaged by pore-forming proteins in bacterial pathogenesis and immune system. His latest achievements in the field include the description of pore forming mechanism by actinoporins, sea anemones cytolytins, membrane interactions of listeriolysin O, a pore forming toxin from bacteria *Listeria monocytogenes*, and effects of human perforin on lipid membranes. He is also a head of the Infrastructural Centre for Molecular Interaction Analysis at the University of Ljubljana,

where they study molecular interactions and are developing novel approaches on how to study protein binding to membranes.

Gregor Anderluh will present his recent data on interactions of proteins from the bacterial pathogens (listeriolysin O) and human immune system (perforin) with model membrane systems. He and his coworkers study molecular details of various steps of pore formation by these molecules that allow damage of lipid membranes and is important for biological activity. Overview of pore forming proteins (toxins) will be provided, together with some latest methodological approaches that he used in his work, with an emphasis on surface plasmon resonance.



# FRANK BERNHARD

Institute of Biophysical Chemistry, Goethe University,  
Frankfurt

## Cell-free production of membrane proteins

Frank Bernhard is leader of the cell-free expression unit at the Centre for Biomolecular Magnetic Resonance at the Goethe University Frankfurt. He is head of an Instruct core centre for cell-free expression and a major focus of his group is the development of technologies for the high quality cell-free production of membrane proteins.

Cell-free expression can be considered as one core technology of the emerging field of Synthetic Biology. Cell-free expression has a clear modular structure composed out of viral transcription systems, pro- or eukaryotic cell lysates and networks of chaperones or chemical stabilizers. Each unit is independent and the individual modules can be combined according to the

specific requirements of expression experiments. During the lecture a short overview of the cell-free expression technology will be given. The importance of additives, stabilizers and solubilizing agents to modulate product quality will be discussed as well as the procedures of getting high yield production of membrane proteins. Also, lipid screening with nanodiscs and the methods used for structural characterization of cell-free expressed membrane proteins (NMR, X-ray crystallography, electron microscopy) will be mentioned in the presentation. The cell-free production of difficult-to-study membrane protein will be demonstrated on the case studies of GPCRs.

# CEITEC Core Facility



## Biomolecular Interactions and Crystallization

**Head of the facility:** prof. RNDr. Michaela Wimmerová, Ph.D.

**Web:** [www.ceitec.cz/z4](http://www.ceitec.cz/z4)

**CF e-mail:** [bic@ceitec.muni.cz](mailto:bic@ceitec.muni.cz)

### Main activities

The core facility provides services leading to structure characterization of biomolecules and to study (bio)molecular interactions in a real time using mainly biosensor and calorimetry-based methods. It is equipped with the instrumentation to set up **crystallization conditions of biomolecules and their complexes** (crystallization screening, optimization and production of crystals for structural determination, crystal plates storage and automatic inspection), **basic characterization of physical properties of the molecules** (analytical ultracentrifugation, dynamic light scattering, CD and fluorescence spectroscopy, differential scanning calorimetry), and to study thermodynamics and/or kinetics of interactions (isothermal titration calorimetry, surface plasmon resonance, CD spectroscopy, analytical ultracentrifugation). The core facility is a part of the **Czech National Affiliated Centre** of INSTRUCT and the member of the **Association of Resources for Biophysical Research in Europe** (ARBRE).

### Information for users

Biomolecular Interactions and Crystallization core facility is available to external users (academia and companies). Czech and international researchers from universities and research institutes interested in accessing core facilities can benefit from support of the **CEITEC – open access project** funded by the Ministry of Education, Youth and Sports of the Czech Republic. Find more information at the core facility official web site ([www.ceitec.cz/z4](http://www.ceitec.cz/z4)) or contact directly the core facility team by email at [bic@ceitec.muni.cz](mailto:bic@ceitec.muni.cz).

# ARBRE



## Web-site:

<https://www.structuralbiology.eu/networks/association-resources-biophysical-research-europe>

The recently created **Association of Resources for Biophysical Research in Europe** (ARBRE) is an open pan-European network, which aims to bring together academic and industrial research infrastructures, core facilities and resource labs that provide access to biophysical instrumentation and expertise for the molecular-scale characterization of biological systems.

The network aims to generate a community focused on broadening expertise and inspiring novel methodological development. It addresses all scientists and technicians who utilize biophysical instrumentation to characterize the intrinsic properties of biological macromolecules and the assemblies in which they are involved.

### The scope of technologies includes (but is not limited to):

- hydrodynamic approaches: analytical ultracentrifugation, light scattering (dynamic and static), SAXS-SANS, thermophoresis, electrophoretic mobility, viscometry, ...
- spectroscopies: fluorescence, infrared, circular dichroism, Raman, electron spin resonance,...
- real-time biosensing based on: surface plasmon resonance, interferometry (DPI, BLI, BSI), QCM, ...
- microcalorimetry, differential scanning fluorimetry,...
- atomic force microscopy, single-molecule approaches, ...
- structural mass spectroscopy, NMR, ...

The initial kick-off meeting took place in June 2014 at the Institut Pasteur (Paris, France). Following this meeting, scientists working within or in close association with **70** facilities/resource labs from 15 European countries have registered as members.

### Minimal criteria for ARBRE membership are:

- \* *provision of access to biophysical instrumentation (and relevant training) for a scientific community.*
- \* *provision of expertise and/or services in the field of molecular-scale characterization of biological systems*
- \* *participation to methodological research driven by biological questions.*

Membership indicates willingness to share openly with the ARBRE community information about available instrumentation and expertise, as well as tips and protocols.

The next ARBRE meeting will take place in London on January 8-9, 2015.

### For further information or membership requests, please contact the ARBRE coordinators:

**Patrick ENGLAND** (Paris, France; <http://www.pasteur.fr/biophysics>): [patrick.england@pasteur.fr](mailto:patrick.england@pasteur.fr)

**Thomas JOWITT** (Manchester, UK; [http://www.wellcome-matrix.org/research\\_groups/thomas-jowitt.html](http://www.wellcome-matrix.org/research_groups/thomas-jowitt.html)): [thomas.a.jowitt@manchester.ac.uk](mailto:thomas.a.jowitt@manchester.ac.uk)

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