will be shown [6]. Our findings also explain sources of inconsistences and irreproducibilities in contemporary biology [7,8].

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Friday, March 20, Session VI

L22

QUANTITATIVE ANALYSIS OF BIOMOLECULAR INTERACTIONS WITH MICROSCALE THERMOPHORESIS (MST)

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Microscale Thermophoresis (MST) allows for quantitative analysis of protein interactions in free solutions and with low sample consumption. The technique is based on thermophoresis, the directed motion of molecules in temperature gradients. Thermophoresis is highly sensitive to all types of binding-induced changes of molecular properties, be it in size, charge, hydration shell or conformation. In an all optical approach, thermophoresis is induced using an infrared laser for local heating, and molecule mobility in the temperature gradient is analyzed via fluorescence. In addition to fluorescence by labels or fusion proteins attached to one of the binding partners, intrinsic protein fluorescence can be utilized for MST thus allowing for label-free MST analysis. Its flexibility in assay design qualifies MST for biomolecular interactionanalysis in complex experimental settings, which we herein demonstrate by addressing typically challenging types of binding events from various fields of life science. The interaction of small molecules and peptides with proteins is, despite the high molecular weight ratio, readily accessible via MST. Furthermore, MST assays are highly adaptable to fit to the diverse requirements of different biomolecules, e.g. membrane proteins to be stabilized in solution. The type of buffer and additives can be chosen freely. Measuring is even possible in complex bioliquids like celllysate and thus under close to in vivo conditions and without sample purification. Binding modes that are quantifiable via MST include dimerization, cooperativity and competition.

L23

INTERDOMAIN INTERACTION IN THE MOTOR SUBUNIT OF THE TYPE I RESTRICTION-MODIFICATION SYSTEM EcoR124I AND THEIR FUNCTIONAL RELEVANCE

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EcoR124I is a type I restriction-modification (R-M) enzyme and as such forms multifunctional heteromeric complexes with DNA cleavage and ATP-dependent DNA translocation activities located on motor subunit HsdR. When unmethylated invading DNA is recognized by the complex, two HsdR endonuclease/motor subunits start to translocate dsDNA without strand separation activity up to thousands base pairs towards the stationary enzyme while consuming ~1 molecule of ATP per base pair advanced. Finally, after translocation is blocked the HsdR subunits cleave the dsDNA nonspecifically far from recognition site. The crystal structure of the motor subunit R determined by our group in 2009 [1] revealed the four domains within the subunit in a square planer arrangement. Computational modeling including molecular dynamics in combination with crystallography, point mutations, in vivo and in vitro assays reveal how interactions between these four domains contribute to ATP-dependent DNA translocation, DNA cleavage or interdomain communication between the translocase and endonuclease activities [2, 3, 4, 5].

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L24

WAVELET FILTER FOR FEMTOSECOND STIMULATED RAMAN SPECTROSCOPY: A NEW APPROACH BRINGS NEW HORIZONS

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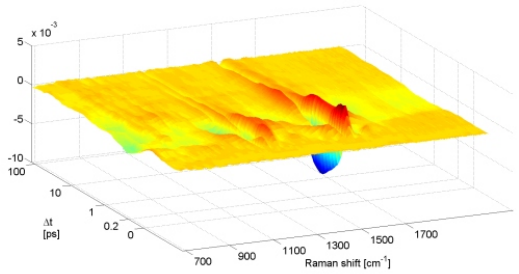
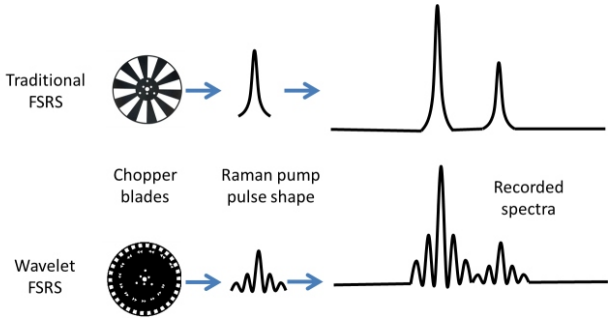
It is now more than 50 years since the discovery of stimulated Raman phenomena and more than 15 years since establishing the femtosecond stimulated Raman spectroscopy (FSRS) technique. Nevertheless, stimulated Raman measurements as an analytical probe have not become a widespread and universal tool, as major problems persisted through unresolved issues of parasitic signals and insufficient sensitivity of applied detection techniques.

Here, we attempt to solve both issues simultaneously by a simple but radical shift in the experimental approach. The traditional way is to generate FSRS signal by a single narrowband picosecond pulse. Such approach is easiest to implement, but in several independent ways not practical for actual Raman data acquisition. We developed a new approach where the FSRS signal is generated by pseudo-random waveforms instead of by a single narrowband pulse. The acquired Raman spectra are then convoluted by the applied waveform, and based on the knowledge of the waveform they can be unambiguously deconvoluted with no loss of spectral resolution. The power of the technique is dual. First, the deconvolution works as a very efficient filter that rejects parasitic signals and offsets automatically and with high fidelity, doing away with human bias in baseline estimation. The second gain is hidden in the



A



B

Figure 1. A: Ultrafast vibrational dynamics of the peridinin-chlorophyll protein (PCP), aphotosynthetic light-harvesting complex of red algae. Each time-resolved FSRS spectrum is the result of only 4 seconds of data accumulation, resulting in baseline noise of ~100μOD. The entire experiment results from only 5 minutes of acquisition time on 1 kHz laser system. B: Wavelet generation through a specially designed chopper blade allows to implement WR-FSRS with minimal extra costs in comparison to traditional FSRS, as no programmable pulse shaper is needed.

delocalization of the signal over a broad interval on the detector array, greatly reducing the so called "fixed pattern noise". In fact, very similar approaches have for decades been a workhorse in many technologies such as Wi-Fi data transfers. With the Wavelet-Resolved FSRS (WR-FSRS) we managed to record femtosecond transient Raman spectra to a sensitivity approaching units of μOD in only tens of seconds of accumulation time. To our best knowledge WR-FSRS increases the resolving power of FSRS experiments at least one order of magnitude while making data processing entirely automatic, rendering the experiment accessible to researchers without FSRS expertise. Based on the data we acquired, we believe that WR-FSRS can be a breakthrough in the field time-resolved Raman spectroscopy.

L25

STRUCTURAL STUDIES OF ASK1-TBD:TRX1 COMPLEX BY FLUORESCENCE SPECTROSCOPY AND SAXS

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family that activates c-Jun N-terminal kinase and p38 MAP kinase pathways in response to various stress stimuli, including oxidative stress, endoplasmic reticulum stress, and calcium ion influx. The function of ASK1 is associated with the activation of apoptosis in various cells and plays a key role in the pathogenesis of multiple diseases including cancer, neurodegeneration and cardiovascular diseases. The kinase activity of ASK1 is regulated by many factors, including binding of thioredoxin (Trx) and the 14-3-3 protein that both function as inhibitors of ASK1 [1]. However, the mechanisms by which these binding interactions inhibit ASK1 are still unclear.

To better understand the role of Trx binding in the inhibition of ASK1, we performed structural characterization of the isolated Trx-binding region of ASK1 (ASK1-TBD) and its complex with reduced TRX1 using fluorescence spectroscopy, circular dichroism and small-angle X-ray scattering. It has been shown that ASK1-TBD is a compact monomeric and rigid domain that under reducing conditions forms with TRX1 a stable and well defined complex with 1:1 molar stoichiometry. We showed that Trp31 of TRX1 is directly involved in the interaction. Moreover, TRX1 interacts with the region of ASK1-TBD located in the vicinity of Cys250 [2].

To elucidate the role of cysteine residues of both proteins in the interaction under reducing and oxidative conditions, we will use spectrophotometric assay with Ellman´s reagent.

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L26

BIOLOGICAL SMALL ANGLE X-RAY SCATTERING AT CEITEC-MU

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Biological Small Angle X-ray Scattering (Bio-SAXS) become mature and popular technique for structural studies of the macromolecules and macromolecular complexes in solution. Development of the software tools available and advances of synchrotron and „home“ X-ray sources brought Bio-SAXS to routine work-flow of number of structural biologists. Bio-SAXS is used for determination of the integral structural parameters, shape reconstruction, determination of the oligomeric and folding state, unraveling the quaternary architecture of the complexes, modeling of molecular flexibility and more. Bio-SAXS characterizes macromolecules in solution, *i.e.* close to their native and biologically relevant conditions. It is a low-resolution technique, but in combination with other techniques as X-ray crystallography, nuclear magnetic resonance, etc., the Bio-SAXS becomes powerful tool of the structural analysis of biological macromolecules.

The Core facility X-ray diffraction and Bio-SAXS of the CEITEC-MU located in Brno facilitates access to the state of art “in house” instrumentation for X-ray structural analysis. Besides the elementary collection of diffraction or scattering data, the facility offers assistance with data processing and interpretation. Year and half after the official opening of the laboratory the most typical Bio-SAXS case studies from users community are presented: *ab initio* shape reconstruction experiments, oligomeric state determination and oligomeric equilibrium studies, hybrid method approaches for quaternary structure model building of macromolecular complexes and studies of semi-flexible complexes and intrinsically disordered proteins.

Friday, March 20, Session VII

L27

THEORETICAL AND EXPERIMENTAL STUDY OF CHARGE TRANSFER THROUGH DNA: IMPACT OF MERCURY MEDIATED T-Hg-T BASE PAIR

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DNA-Hg complexes may play an important role in sensing of DNA defects or in detecting of Hg presence in the environment. A fundamental way of characterizing DNA-Hg complexes is to study the way how the electric charge is transferred through the molecular chain. The main goal of this contribution was to investigate the impact of a mercury metal cation that links two thymine bases in a DNA T-T mismatched base pair (T-Hg-T) on charge transfer through the DNA molecule. We compared the charge transfer efficiencies in standard DNA, DNA with mismatched T-T base pairs and DNA with T-Hg(II)-T base pair. For this purpose we measured the temperature dependence of steady-state fluorescence and UV-VIS of the DNA molecules. The experimental results were confronted with the results obtained employing theoretical DFT methods. Generally, the efficiency of charge transfer was driven by mercury changing the spatial overlap of bases.