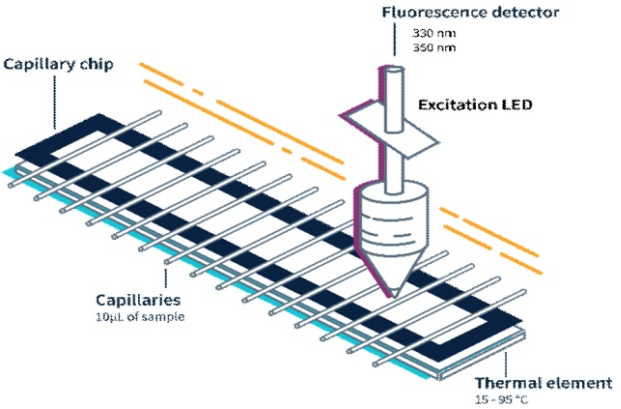
measured in solution, and hard-to-measure viscous samples are examined with no problem.

Experiments performed with Prometheus platform deliver $T_m$, $T_{onset}$ and $T_{agg}$ , $C_m$, G and G as well as time resolved parameters in isothermal conditions.

Our Prometheus allows for parallel measurement of protein aggregation propensity of tested samples, providing valuable information on their colloidal state.

All above make nanoDSF, and specifically Prometheus platform, the method of choice for easy, fast and precise analysis of protein folding and stability.

On the practical note; Prometheus does not require maintenance, flushing, equilibration or any other between-experiments interventions, making it money saving and multi users- friendly, as samples solutions do not come in direct contact with the instrument.




**To discuss your specific research interest we invite you to visit our company booth during the meeting.**

## Friday, March 20, Session III

**L10**

# MODULATING FOXO3 TRANSCRIPTIONAL ACTIVITY BY SMALL, DBD-BINDING MOLECULES

**K. Kohoutova[1,4], V. Docekal[2], J. Vesely[2], M. J. Ausserlechner[3], V. Obsilova[4], T. Obsil[1]**

*[1]Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Hlavova 8, Prague 2, 12843¨*
*[2]Department of Organic Chemistry, Faculty of Science, Charles University, Hlavova 8, Prague 2, 12843*
*[3]Department of Pediatrics I, Medical University Innsbruck, Innsbruck, Austria*
*[4]Department of Structural Biology of Signaling Proteins, Biotechnology and Biomedicine Center of the Academy of Sciences and Charles University in Vestec (BIOCEV), Institute of Physiology, Czech Academy of Sciences, 14220 Prague, Czech Republic*
*kohoutk6@natur.cuni.cz*

FOXO3 is a member of FOXO Transcription Factor family. FOXO proteins share a conserved DNA-binding motif called winged-helix DNA-binding domain (DBD). FOXO3 recognizes specific DNA sequence. Thourgh interaction with target DNA it modulates various biological processes, such as cell death, cell-cycle arrest, DNA repair and energy homeostasis [1] . Due to its ability to induce cell cycle arrest it is considered a tumor suppressor. However, in certain cases, it has been shown that FOXO3 promotes tumor development and angiogenesis via maintaining cancer cell energy homeostasis. FOXO3 can also enhance tumor cell resistence to chemoterapeutic agents [2]. Therefore, targeting of FOXO3 transcriptional activities by specific inhibitors can help to prevent drug resistance in cancer therapy.

A pharmacophore screening identified a small molecule compound that physically interacts with the FOXO3-DBD and modulates the FOXO3 transcriptional program in human cells. The mode of interaction between this compound and the FOXO3-DBD was characterized by


**Figure 1 – Graphical scheme of abstract**

NMR spectroscopy and docking studies [3] . This compound was further modified to increase its inhibitory potency. In this work we tested a group of newly designed inhibitors of FOXO3 transcriptional activity. Their inhibitory potency and interaction with FOXO3-DBD was tested using NMR and native electrophoresis. Furthermore, the effect of these compounds on FOXO3 transcriptional activity was evaluated in cell cultures. We have shown that these new derivatives are able not only to bind to FOXO3-DBD but also to inhibit its interaction with the target DNA.

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**L11**

## INVESTIGATION INTO G4–LIGAND COMPLEX IN LIVING HUMAN CELLS USING IN-CELL NMR SPECTROSCOPY

**Daniel Krafčík[1,3,*], Michaela Krafčíková[2], Eva Maturová[1], Pavlína Víšková[1], Kateřina Beková[1], Silvie Foldynová-Trantírková[1,2], Lukáš Trantírek[1]**

*[1]Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00, Brno, Czech Republic*
*[2]Institute of Biophysics of the AS CR, v.v.i., Královopolská 135, 612 65, Brno, Czech Republic*
*[3]National Centre for Biomolecular Research, Masaryk University, Brno, Czech Republic*
** krafcik.daniel@gmail.com*

Recently, we established an in-cell NMR based approach for monitoring of interactions between double-stranded DNA targets and low molecular weight compounds (ligands) in living human cells [1, 2]. The method relies on the acquisition of NMR data from cells electroporated either with preformed DNA-ligand complexes or by incubation of electroporated cells with given ligand. The impact of the intracellular environment on the integrity of the complexes is assessed based on in-cell NMR signals from unbound and ligand-bound forms of a given DNA target.

Here, we report how the interactions of chosen examples of G-quadruplex DNA and G-quadruplex binding ligands from the study can be monitored by using the in-cell NMR. This comparative study is based on the data from in vitro measurements, interactions of the complex in the environment of the crude lysate and the in-cell NMR experiments. Our data suggest that in-cell NMR can be used to assess selectivity of G-quadruplex binding ligands with respect to topologically distinct G-quadruplex targets under complex physiological conditions with many times different result than in vitro environment.

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L12

## MOLECULAR MECHANISM OF LEDGF/P75 DIMERIZATION

**Vanda Lux [1], Tine Brouns [2], Kateřina Čermáková [1,3], Pavel Srb [1], Milan Fábry [4], Marcela Mádlíková [1], Magdalena Hořejší [4], Petr Novák [5], Michael Kugler [1], Jiří Brynda [1,4], Zeger Debyser [2], and Václav Veverka [1]**

*[1]Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic*
*[2]KU Leuven, Molecular Virology and Gene Therapy, Leuven, Flanders, Belgium*
*[3]Department of Molecular & Cellular Biology, Center for Precision Environmental Health, and Dan L Duncan Comprehensive Cancer Center, Baylor College of Medicine, Houston, TX, USA*
*[4]Institute of Molecular Genetics of the CAS, Prague, Czech Republic*
*[5]Institute of Microbiology of the CAS, Prague, Czech Republic*
*[6]Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic*
*vanda.lux@uochb.cas.cz*

Protein dimerization of many eukaryotic transcription regulatory factors is critical for regulation of their functional complexes. Recently it was shown, that transcription regulatory role of an epigenetic reader Lens Epithelium Derived Growth Factor/p75 (LEDGF/p75, also known as PSIP1) requires at least two copies of this protein to overcome the nucleosome-induced barrier to transcription elongation. Moreover, various LEDGF/p75 binding partners are enriched for dimeric features further underscoring functional regulatory role of LEDGF/p75 dimerization. Here, we used a combination of biophysical and biochemical techniques to investigate the mechanism of LEDGF/p75 dimerization and its effect on molecular interactions with other proteins. We dissected the minimal dimerization region in the C-terminal part of LEDGF/p75 and with the help of paramagnetic NMR spectroscopy identified the key molecular contacts that were used to refine the solution structure of the dimer. The LEDGF/p75 dimeric assembly is stabilized by domain-swapping within the integrase binding domain and additional electrostatic 'stapling' of the negatively charged -helix formed in the intrinsically disordered C-terminal region. We validated mechanism of dimer formation using structure-inspired dimerization defective LEDGF/p75 variants and chemical cross-linking coupled to mass spectrometry. We also show how dimerization might impact the LEDGF/p75 interactome.

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L13

## STUDY OF THE I-MOTIF STABILITY DURING THE CELL CYCLE PROGRESSION BY IN-CELL NMR SPECTROSCOPY

**Viskova Pavlina [1], Maturova Eva [1], Krafcikova Michaela [1,2], Dzatko Simon [1], Loja Tomas [1], Rynes Jan [1], Mergny Jean-Louis [2,3,4], Foldynova-Trantirkova Silvie [1,2], Trantirek Lukas [1,2]**

*[1] Central European Institute of Technology, Masaryk University, Brno, Czech Republic*
*[2] Institute of Biophysics of the AS CR, v.v.i., Brno, Czech Republic*
*[3]CNRS UMR9187, INSERM U1196, Université Paris Sud, Université Paris Saclay, Orsay 91405, France*
*[4]CNRS UMR9187, INSERM U1196, Insitut Curie, PSL Research University, Orsay 91405, France*
**pavlina.viskova@ceitec.muni.cz*

I-motifs are non-canonical four-stranded DNA structures formed within cytosine (C)-rich DNA [1]. Under *in vitro* conditions, the formation of these structures strongly depends on pH and ionic strength [2, 3]. While the i-motifs readily form under *in vitro* conditions of acidic pH and low ionic strength, their formation propensity at close to physiological conditions is compromised. Yet, the i-motifs exist *in vivo* [5]. Recently, their active role in the regulation of gene expression and telomeric maintenance has been proposed [6].

Cell cycle progression is accompanied by changes in both intracellular pH and ionic strength [4]. As recently demonstrated by Zeraati et al., the *in vivo* occurrence of the i-motifs changes during the cell cycle [7]. Whether the i-motif formation and the i-motif regulated gene expression is coupled with reorganization of the chromatin or whether it is a consequence of the fluctuations in the environmental parameters of the cell, such as pH or ionic strength, remains unclear.

*Here, we report on investigations of formation of the i-motifs in separate phases of the cell cycle using non-invasive in-cell NMR spectroscopy.*

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