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## HIGH-RESOLUTION PROTEIN STABILITY ANALYTICS USING NANODSF

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*NanoTemper Technologies GmbH*

The fluorescence of tryptophans in a protein is strongly dependent on its close surroundings. By following changes in fluorescence, chemical and thermal stability can be assessed in a truly label-free fashion. The dual-UV technology by NanoTemper allows for rapid fluorescence detection, providing an unmatched scanning speed and data point density. This yields ultra-high resolution unfolding curves which allow for detection of even minute unfolding signals. Furthermore, since no secondary reporter fluorophores are required, protein solutions can be analyzed independent of buffer compositions, and over a concentration range of 150 mg/ml down to 5 µg/ml. In

addition, information on protein aggregation can be recorded in parallel, providing insight into colloidal stability of the sample. Therefore, nanoDSF is the method of choice for easy, rapid and accurate analysis of protein folding and stability, with applications in membrane protein research, protein engineering, formulation development and quality control. The presentation will cover biophysical concepts of the technique showing benefits of the nanoDSF technology platform, and will be followed by specific examples of nanoDSF applications towards various experimental systems.

**Tuesday, June 27, Session V**

PL2

## INTEGRATED STRUCTURAL BIOLOGY: GENERAL CONCEPTS AND CASE STUDIES

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## AN INTEGRATIVE STRUCTURAL APPROACH TO RNA:PROTEIN INTERACTIONS

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The detailed characterization of biomolecular interactions requires the combination of different biophysical methods. The concept of integrative structural biology will be illustrated by the structural and functional study of ubiquitous RNase P enzymes, which catalyze the 5' maturation of pre-tRNAs. For a long time it has been thought that all RNase P were ribozymes. However, a novel kind of RNase P composed of proteins only, called PRORP for "Protein-only RNase P" was first discovered in human mitochondria, then described in *Arabidopsis thaliana* (1,2). The latter possesses three PRORP homologs: PRORP1 located in mitochondria and chloroplasts, PRORP2 and PRORP3 in the nucleus.

We developed an integrative approach in order to gain an insight into *Arabidopsis* PRORP enzymes and their mode of action (3). The affinity constant between a mini-

mal tRNA substrate and a catalytically inactive PRORP2 enzyme was first determined by microscale thermophoresis (MST), ultracentrifugation and calorimetry (ITC), and shown to be in the micromolar range. A combination of mutagenesis and affinity measurements helped define the respective importance of individual pentatricopeptide repeats (PPR) of PRORP2 for RNA binding. A comparison of the crystal structure of PRORP2 and of solution structures of the enzyme and its complex with a pre-tRNA obtained by small angle X-ray scattering (SAXS) indicated that PRORP2 undergoes structural changes to accommodate its substrate. A dedicated SAXS setup was implemented to stabilize the complex during analysis. Altogether this study reveals the structural diversity and plasticity of protein-only RNase P enzymes.

1. Holzmann et al. RNase P without RNA: identification and functional reconstitution of the human mitochondrial tRNA processing enzyme. *Cell* (2008), 135, 462-74.
2. Gobert et al. A single Arabidopsis organellar protein has RNase P activity. *NSMB* (2010), 17, 740-4.
3. Gobert et al. Structural insights into protein-only RNase P complexed with tRNA. *Nat Commun* (2013), 4, 1353.

**L16**

## CHARACTERIZATION OF DNA-BINDING PROTEINS

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All organisms use a plethora of proteins interacting with DNA. They fulfill essential roles during cell homeostasis and proliferation. The functions of DNA-binding proteins are diverse and reach from modifying, labeling, checking, processing, regulating, opening, locking, shielding, degrading, editing, transcribing to structuring DNA. Studying DNA-binding proteins is often a challenging task as their function and abundance is tightly controlled, and usually restricted to certain conditions, functional states or cell cycle stages. However, remarkable findings about their cellular roles can be discovered when taking the properties and functional requirements of DNA-binding proteins into

account. The interaction with DNA facilitates additional strategies for the purification of DNA-binding proteins and it offers many methods for their functional characterization. The presentation will focus on special features of DNA-binding proteins and techniques for their functional characterization. In addition, various ways of forming, stabilizing and analyzing protein-DNA complexes will be discussed. Understanding the mode of action of proteins interacting with DNA is crucial not only to comprehend complex biological processes but also to shed light onto their defects and roles in diseases like cancer.

## Wednesday, June 28, Session VI

**L17**

## FROM PROTEIN CHEMICAL MODIFICATION TO CROSS-LINKING AND BEYOND

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Even the first protein structural models were built using X-ray crystallography and NMR spectroscopy sixty and thirty years ago, respectively. There are still many protein sequences and protein complexes with unknown 3-D structure. The tremendous progress in mass spectrometry in last decades opened the space for studying protein folding, protein/ligand interactions and protein dynamics in solution. The potential of ion mobility, chemical cross-linking and

hydrogen/deuterium exchange for structural biology will be discussed.

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