Friday, March 14, Session IV

L16

MICROSCALE THERMOPHORESIS: INTERACTION ANALYSIS AND BEYOND

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MicroScale thermophoresis (MST) is an exceptionally powerful technique to quantify biomolecular interactions. It is based on thermophoresis, the directed movement of molecules in a temperature gradient, which strongly depends on a variety of molecular properties such as size, charge, hydration shell or conformation. Thus, this technique is highly sensitive to virtually any change in molecular properties, allowing for a precise quantification of molecular events independent of the size or nature of the investigated specimen.

During an MST experiment, a temperature gradient is induced by an infrared laser. The directed movement of molecules through the temperature gradient is detected and quantified using either covalently attached or intrinsic fluorophores. By combining the precision of fluorescence detection with the variability and sensitivity of thermophoresis, MST provides a flexible, robust and fast way to dissect molecular interactions.

In this work, we present recent progress and developments in MST technology and focus on MST applications beyond standard biomolecular interaction studies. By using different model systems, we introduce alternative MST applications – such as determination of binding stoichiometries and binding modes, analysis of protein unfolding, thermodynamics and enzyme kinetics – and also demonstrate the capability of MST to quantify high-affinity interactions with K_ds in the low pM range as well as protein-protein interactions in pure cell lysates.

L17

RAMAN MICROSPECTROSCOPY OF THE YEAST VACUOLES

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Extensive effort was undertaken recently to understand pathogenicity and virulence of some *Candida* species [1]. Their ability to escape immune defense of the host and survive even under the severe nutrition limitations and in the presence of various stress factors or antimycotics seems to be related, among others, to preservation of their vacuolar functions. Non-invasive methods providing information about chemical composition of the vacuoles within living cells exposed to various external factors are thus of great importance for development of novel antifungal strategies.

Especially, concentration and structural properties of polyphosphates accumulated in the yeast vacuoles are of particular interest, since they are sensitive to nutritional limitations and environmental stresses, and can thus reflect physiological state of the cell [2]. Recently, Raman microspectroscopy was suggested as practical alternative to laborious and time-consuming chemical analysis of intracellular polyphosphate inclusions in polyphosphate-accumulating bacteria [3]. In the present work, the method was used to monitor polyphosphates and other chemical compounds dissolved in the vacuoles of living *Candida* yeasts of different physiological state and/or under various stress conditions. It was shown that using proper immobilization protocols, the spatially-resolved Raman spectra from individual yeasts throughout statistically sound sets of living cells can be collected routinely. Abundance distributions of actual concentration and polymerization degree of polyphosphates can be assessed employing advanced multivariate methods for spectral analysis of structurally sensitive polyphosphate bands (~688 and ~1154 cm^{-1}) properly normalized with respect to the water OH stretches at 3400 cm^{-1} [4].

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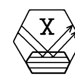

L18

THEORETICAL AND EXPERIMENTAL STUDY OF CHARGE TRANSFER THROUGH DNA: IMPACT OF MERCURY ATTACHED TO MISMATCHED BASE PAIRS

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DNA-Hg complexes play an important role in sensing of defects in DNA or presence of Hg in the environment. A fundamental way of characterizing DNA-Hg complexes is investigating the way electric charge is transferred through the complex. The main goal of this contribution was to investigate the impact of mercury metal cation that links two thymine bases in DNA T-T mismached 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. In our case it was namely the spatial overlap of bases that substantially influenced the calculated charge transfer rates, and the overlap of bases was notably affected by the presence of Hg(II) linkage in the T-T mismatch. Our investigation of the metallo DNA duplex provides the basis for design of metal-conjugated nucleic acid nanomaterials.

L19

STRUCTURE, STABILITY AND THERMODYNAMIC CHARACTERISTICS OF SINGLE STRANDED DNA IN REPETITIVE EXTRAGENIC PALINDROMIC ELEMENTS

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Repetitive extragenic palindromes (REPs) are small imperfect palindromic repeats of 20–40 DNA nucleotides in length which are found in many bacterial species at a high copy number [1]. They are important in the regulation of certain bacterial functions, such as Integration Host Factor recruitment and mRNA turnover. REPs consist of a GTAG tetranucleotide and a nearby self-complementary GC-rich sequence with the potential to form stable non canonical DNA secondary structures. Single strand hairpin stem loop conformations have been shown to provide preferable binding sites for the REP-associated tyrosine transposase (RAYT) from *Escherichia coli* [2]. The conserved tetranucleotide and palindrome of REP elements play a key role in REP recognition by RAYTs.

Genomic sequence analysis has identified numerous potential REPs elements in a number of different host bacterial species. REPs from Escherichia coli, Stenotrophomonas maltophilia and Cardiobacterium hominis were chosen to explore the possibility of non-canonical DNA structure formation in these sequences similar to that observed for REP of Escherichia coli. Spectroscopic and calorimetric melting studies of single stranded oligonucleotide REP sequences were performed to determine their conformation, stability and thermodynamic characteristics.

Stability of oligonucleotides conformations was ascertained from their temperature-induced melting transitions monitored by ultraviolet absorbance. Model dependent transition parameters were estimated from a van't Hoff analysis of the melting curves and, in select cases, compared to model free calorimetric measurements. The conformational state of the oligonucelotides was assessed by circular dichroism spectroscopy (CD) and the concentration dependence of the melting temperature. Our data showed that oligonucleotides corresponding to REP recognition DNA sequences from different bacteria can adopt a range of non-canonical DNA conformations in solution,

which may be important for RAYT recognition of REP elements.

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L20

RSL LECTIN - A COMPLEX APPROACH TO STUDY CARBOHYDRATE-PROTEIN INTERACTION

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Lectins, carbohydrate-binding proteins, play an important role in various processes like cell-cell communication or host-pathogen interaction. Due to their specificity and reversibility in binding, they are also widely used for detection, separation and/or analysis of various glycans and glycoproteins [1]. The detailed understanding of interaction between the protein and particular saccharide may help both in analyzing lectin function and lectin engineering for scientific applications.

Fucose-binding lectin AAL from *Aleuria aurantia* is known for decades and widely used in research [2]. However, its five non-equivalent binding sites make it an uneasy target for detailed analysis of protein-ligand interaction. Its structural homologue RSL form *Ralstonia solanacearum*, on the other hand, forms only two sets of binding site of very similar amino acid composition [3]. As such, this lectin can be a suitable model for AAL, or maybe even replace it in some applications.

In our study, we focused mainly on RSL ability to bind - and -methylfucoside. We created a set of mutants in order to distinguish between both RSL binding sites. Several methods were combined to analyze the interaction of the proteins with saccharides from the thermodynamical and structural point of view. Hence we employed isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) titration as well as X-ray crystallography, where we obtained several high-resolution structures. Combining all these methods, we got a complex view on lectin-carbohydrate interaction in this interesting lectin family.

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